



US009051586B2

(12) **United States Patent**
Cha et al.

(10) **Patent No.:** **US 9,051,586 B2**
(45) **Date of Patent:** **Jun. 9, 2015**

(54) **METHOD FOR CONVERTING AND PRODUCING CARBONATE MINERALS FROM CARBON DIOXIDE USING RECOMBINANT BIOCATALYST**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 61 days.

(21) Appl. No.: **13/817,468**

(22) PCT Filed: **Apr. 13, 2012**

(86) PCT No.: **PCT/KR2012/002816**

§ 371 (c)(1),

(2), (4) Date: **Feb. 18, 2013**

(87) PCT Pub. No.: **WO2013/002481**

PCT Pub. Date: **Jan. 3, 2013**

(65) **Prior Publication Data**

US 2014/0113339 A1 Apr. 24, 2014

(30) **Foreign Application Priority Data**

Jun. 29, 2011 (KR) 10-2011-0063729
Mar. 7, 2012 (KR) 10-2012-0023429

(51) **Int. Cl.**

C12P 9/00 (2006.01)
C12P 7/00 (2006.01)
C12P 7/62 (2006.01)
C12N 9/88 (2006.01)
C12N 1/20 (2006.01)
A61L 9/01 (2006.01)
C12P 21/06 (2006.01)
C12P 19/34 (2006.01)
C07H 21/04 (2006.01)
C07K 1/00 (2006.01)
C12P 3/00 (2006.01)

(52) **U.S. Cl.**

CPC ... **C12P 9/00** (2013.01); **C12N 9/88** (2013.01);
C12P 3/00 (2013.01)

(58) **Field of Classification Search**

CPC **C12N 9/88**
USPC 435/131, 132, 135, 232, 252.3, 252.33,
435/266, 69.1, 91.1; 536/23.1, 23.2;
530/350

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to a technique for capturing carbon dioxide and converting the carbon dioxide to carbonate minerals using a recombinant whole cell biocatalyst expressing carbonic anhydrase. More particularly, the present invention relates to a composition for capturing carbon dioxide and a method for capturing carbon dioxide using the composition, which composition comprises a whole cell of a transformant formed with a vector including a nucleic acid encoding a recombinant carbonic anhydrase; a cell lysate or its fraction of the whole cell; or a recombinant carbonic anhydrase isolated from the whole cell. Further, the present invention relates to a composition and method for converting the carbon dioxide to carbonate minerals using the carbon dioxide capturing composition.

14 Claims, 13 Drawing Sheets
(11 of 13 Drawing Sheet(s) Filed in Color)

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Figure 1

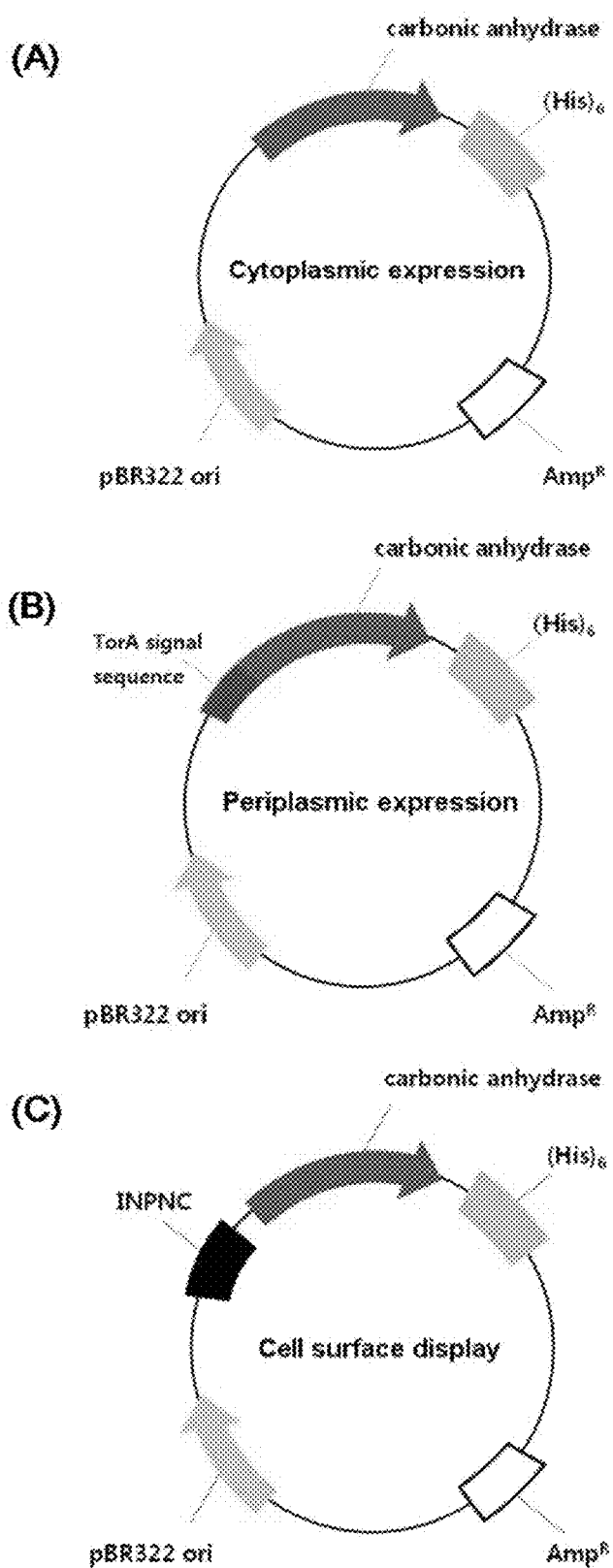


Figure 2

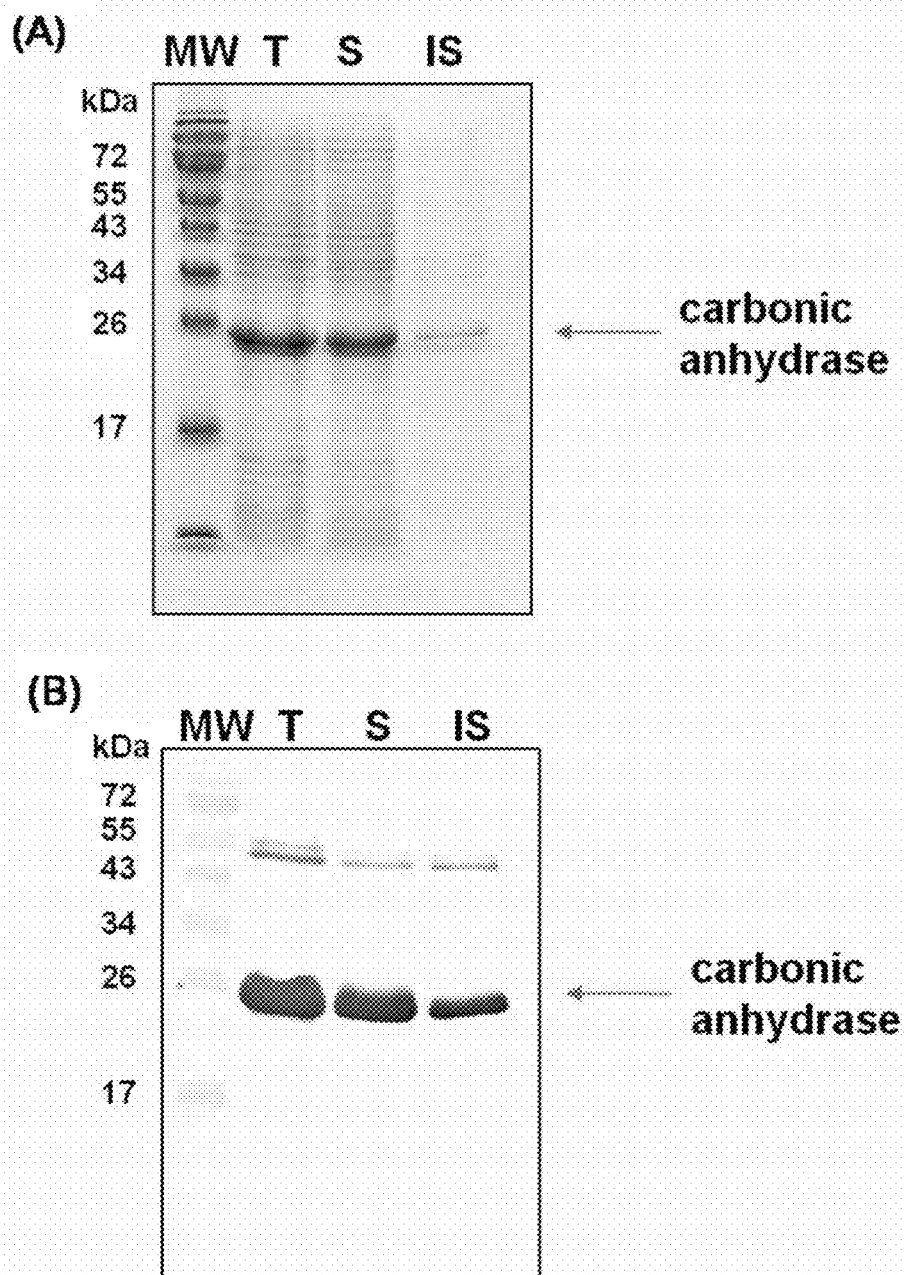


Figure 3

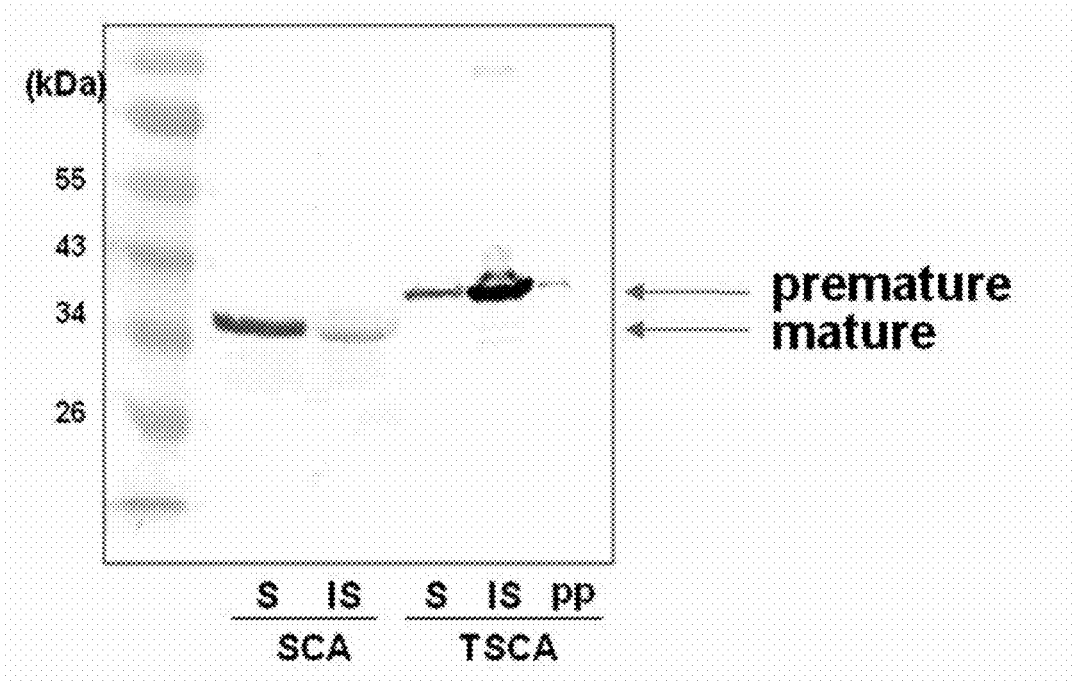


Figure 4

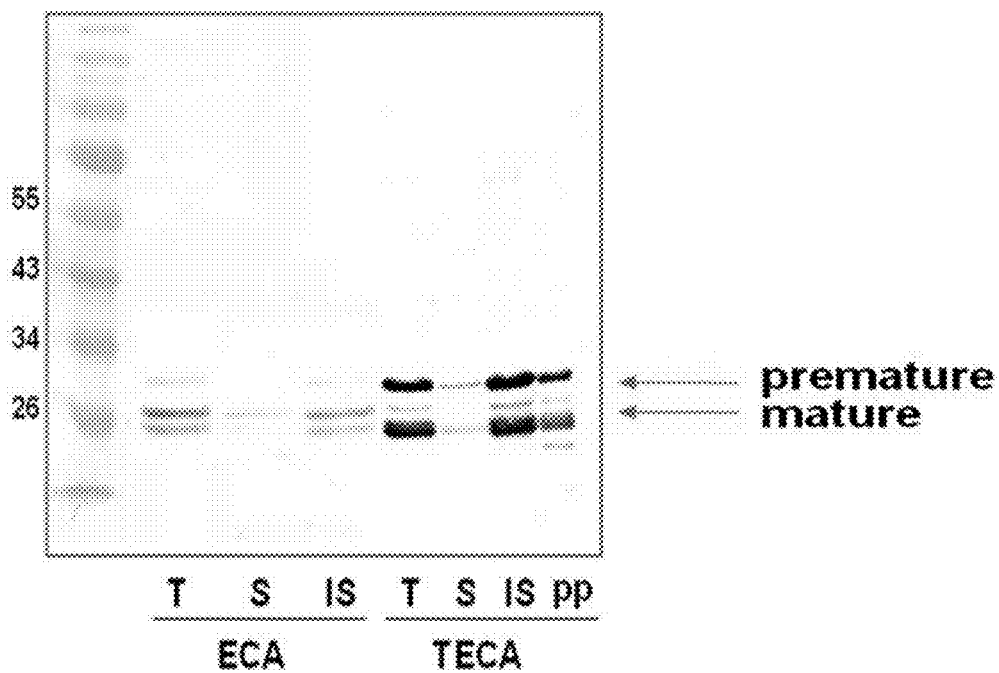


Figure 5

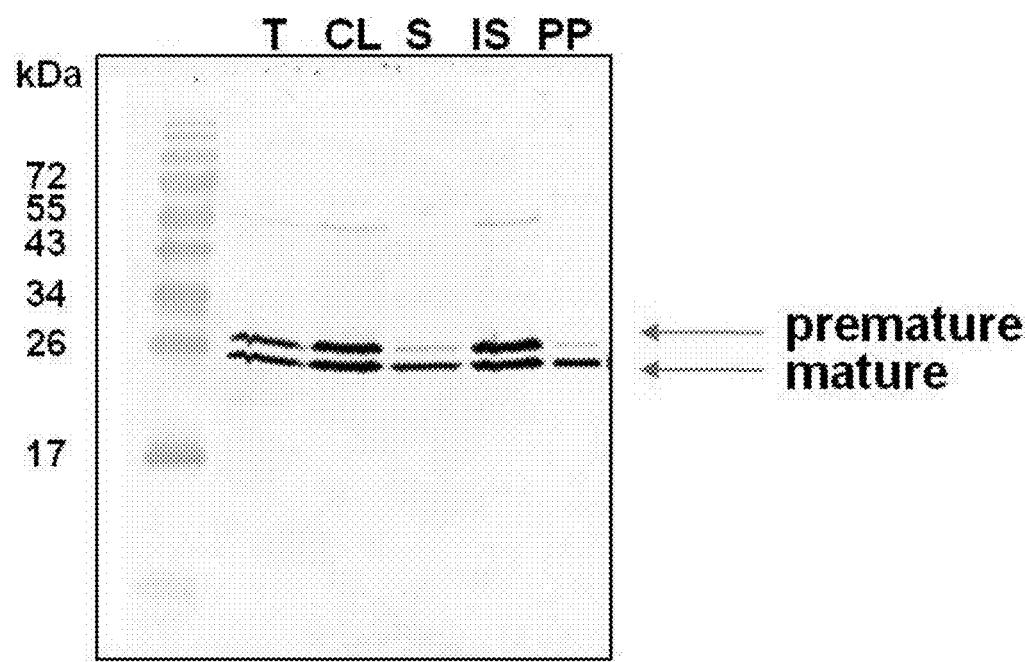


Figure 6

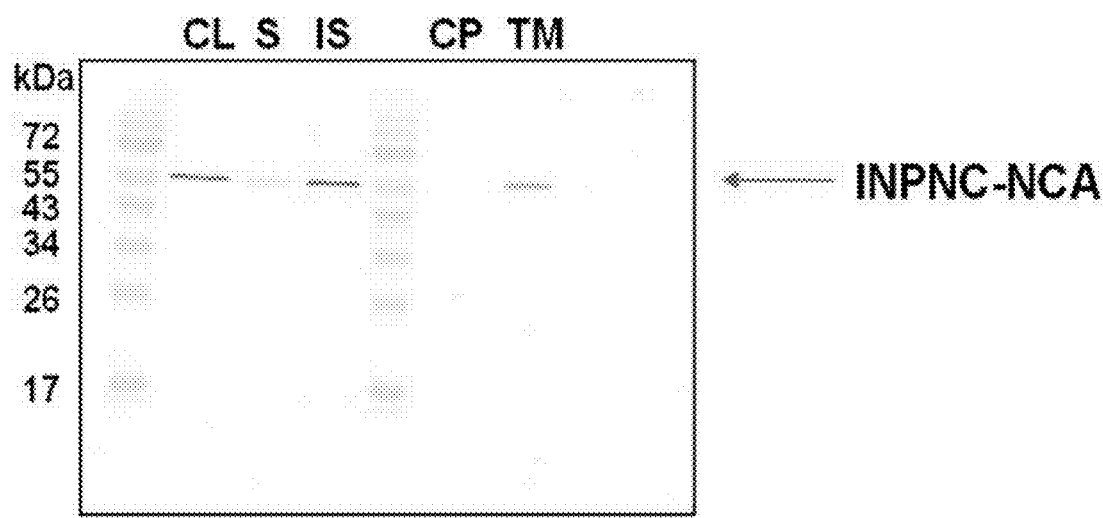


Figure 7

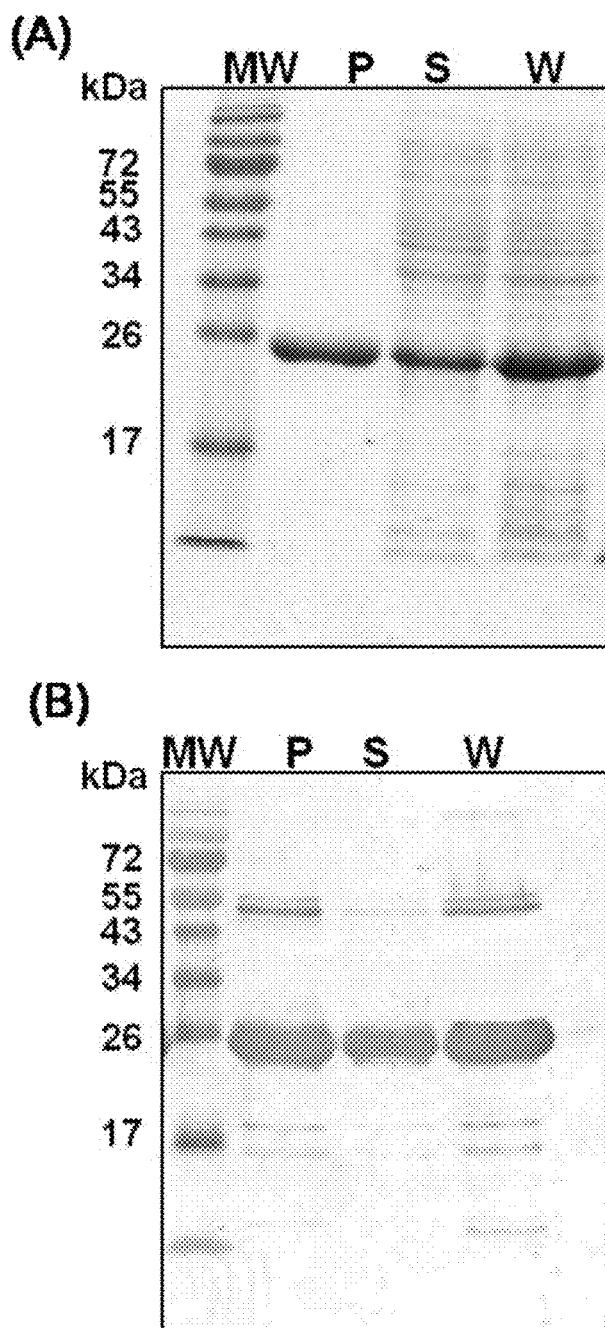
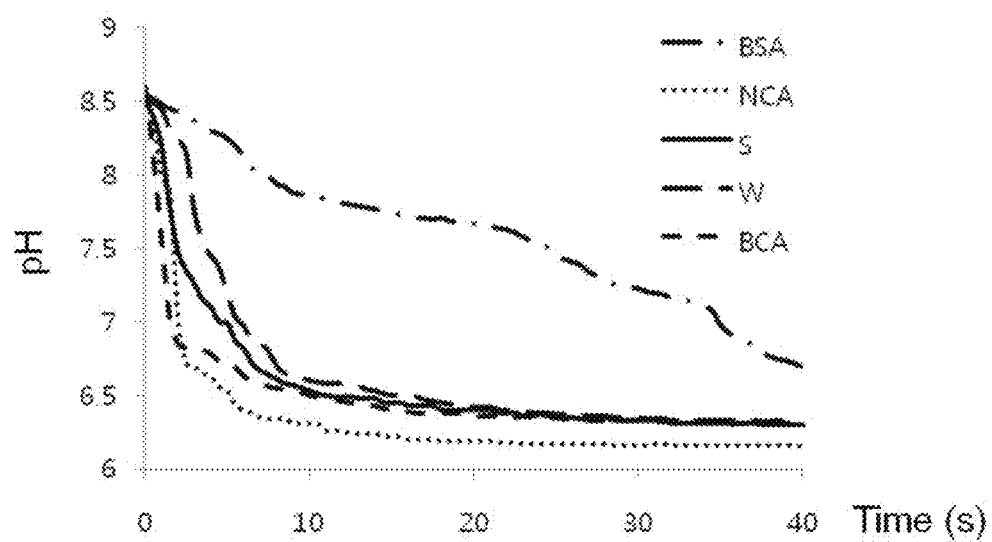


Figure 8



	BCA	NCA	S	W
Time(s)	0.7	0.98	2.25	2.75
Activity (U/mg)	3090	2184	920	728

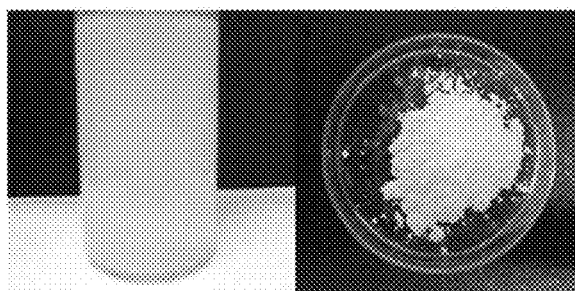
Figure 9



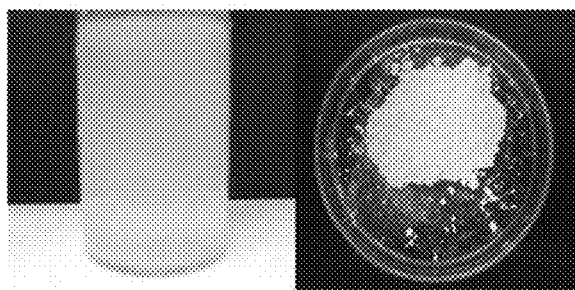
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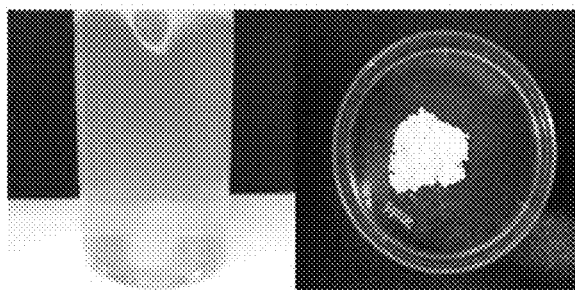
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Figure 10

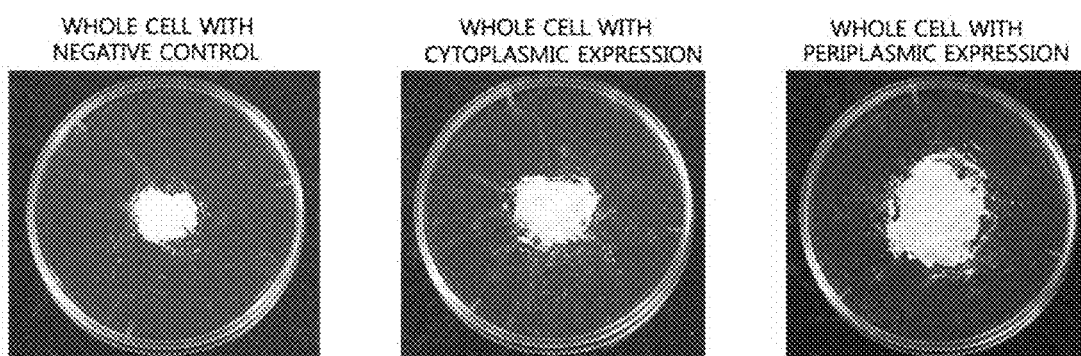


Figure 11

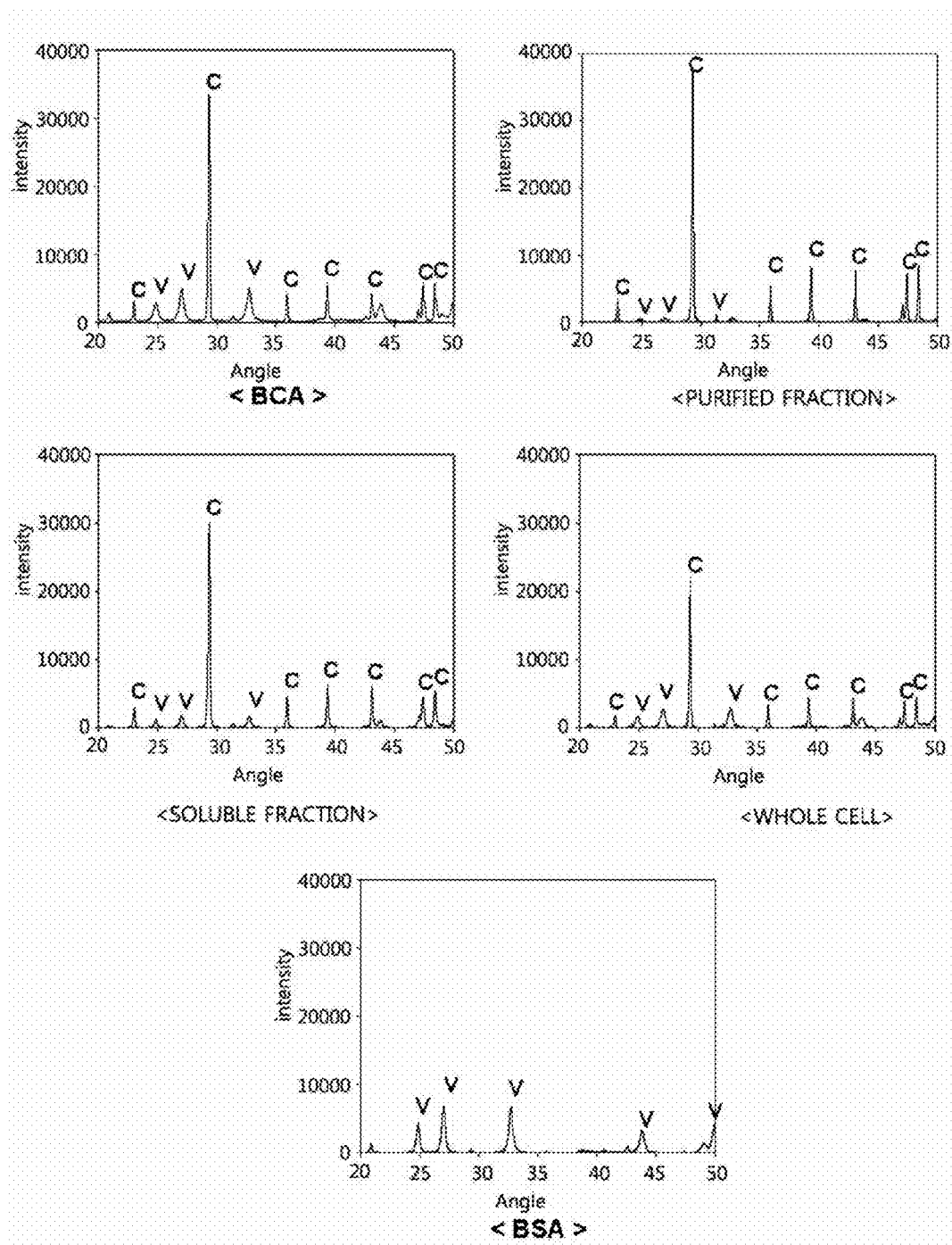


Figure 12

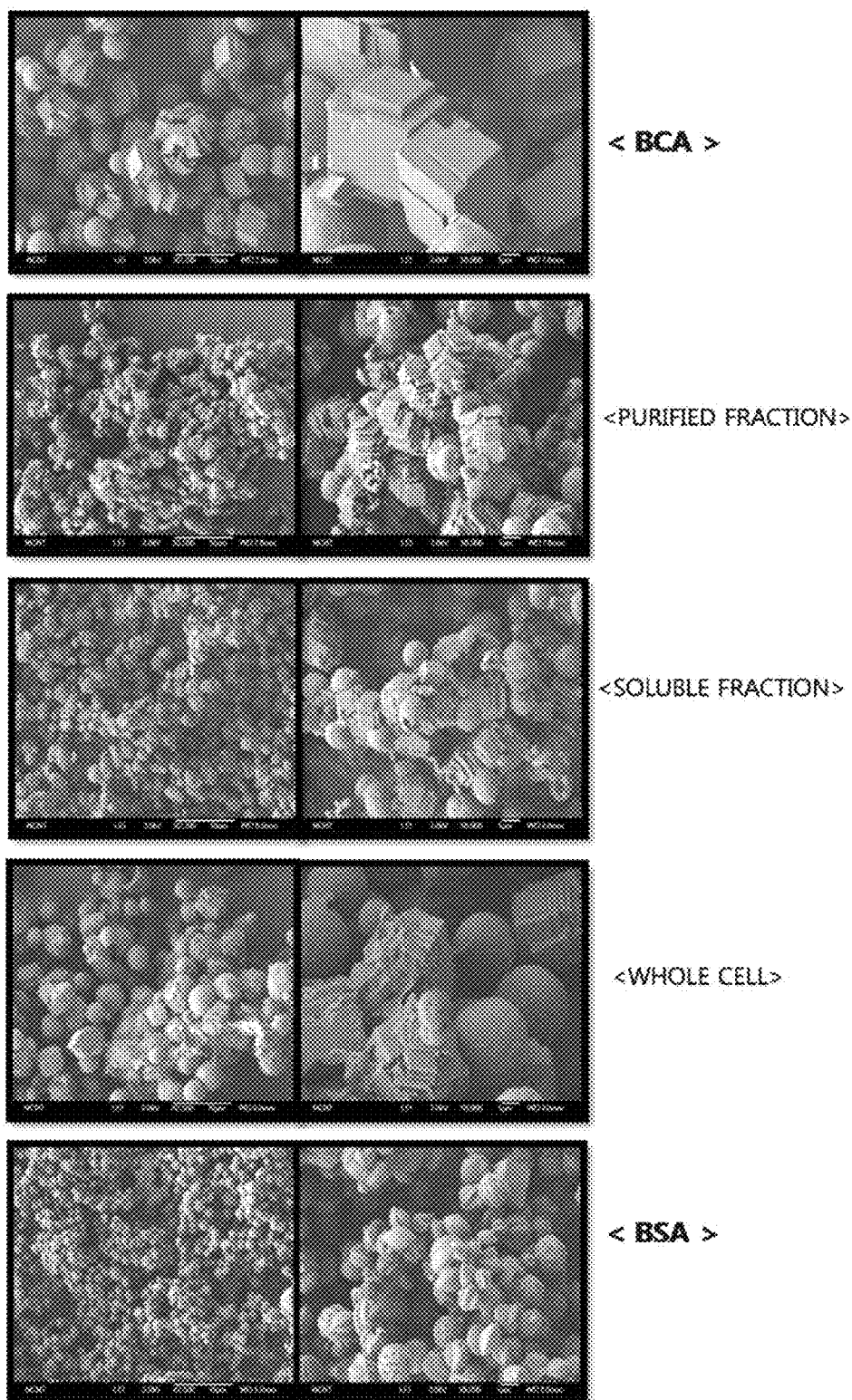
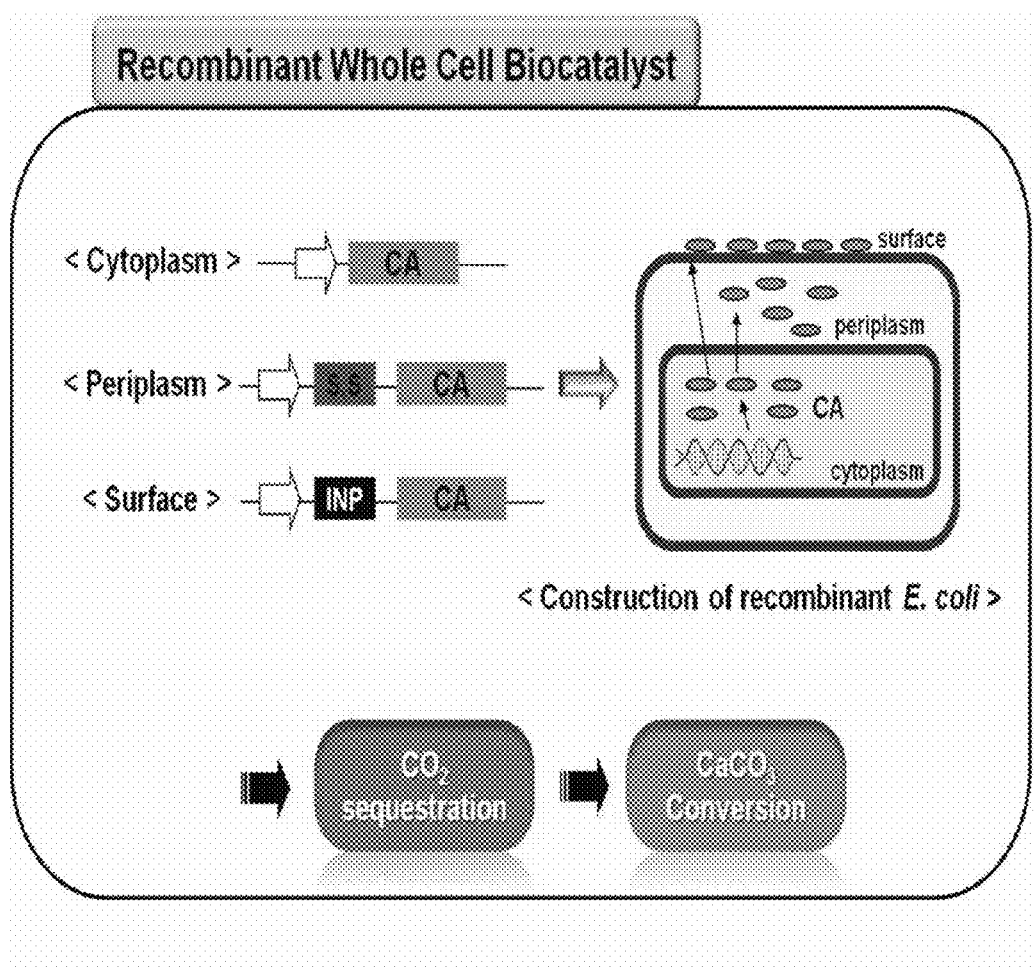


Figure 13



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METHOD FOR CONVERTING AND PRODUCING CARBONATE MINERALS FROM CARBON DIOXIDE USING RECOMBINANT BIOCATALYST

TECHNICAL FIELD

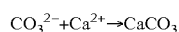
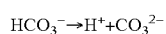
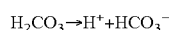
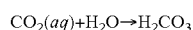
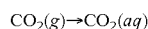
The present invention relates to a technique for capturing carbon dioxide and converting the carbon dioxide to carbonate minerals using a recombinant whole cell biocatalyst expressing carbonic anhydrase. More particularly, the present invention relates to a composition for capturing carbon dioxide (CO₂) and a method for capturing carbon dioxide using the composition, which comprises: a recombinant whole cell transformed with a vector containing a nucleic acid encoding a carbonic anhydrase to express a carbonic anhydrase in cytoplasm, periplasmic space, or cell surface; a cell lysate or its fraction of the whole cell; or a recombinant carbonic anhydrase isolated from the whole cell. Further, the present invention relates to a composition and method for converting the carbon dioxide to carbonate minerals using the composition for capturing carbon dioxide.

BACKGROUND ART

There have been increasing worldwide attempts to reduce the concentration of carbon dioxide (CO₂) in the atmosphere in association with the global warming issue. The establishment of techniques for reducing carbon dioxide is necessary in consideration of the current situation that the use of fossil energy is going to be inevitable in near future in spite of the ongoing development of renewable energy. A variety of chemical and physical methods for absorption of carbon dioxide have been developed and mostly encounter problems related to high heat recovery, corrosion, additional storage space, and so forth. Recently, attractions have been drawn by a method for a biological capture of carbon dioxide (CO₂) with an enzyme involved in biological CO₂ fixation. Such a method for biological CO₂ capture is of great benefit in the aspects of eco-friendliness, rapid reaction, and above all, conversion of carbon dioxide to the final compound, more advantageous over the conventional chemical and physical methods.

Carbonic anhydrase is a Zn-containing metalloenzyme that is known to exist in tissues of mammals, plants, or green algae and catalyze the hydration of carbon dioxide. Up to date, the carbonic anhydrase has been classified into five categories according to sequence similarity: α , β , γ , δ , and ϵ . For example, α -carbonic anhydrase is the type to be found in most of mammals, and part of bacteria and green algae; β -carbonic anhydrase is present in most of prokaryotes and plants; γ -carbonic anhydrase is found in methane-producing bacteria, *Methanosarcina thermophila*; δ -carbonic anhydrase is a recently reported carbonic anhydrase found in diatoms; and ϵ -carbonic anhydrase is present in part of chemolithotrophs.

Carbon dioxide in atmosphere is dissolved in water to form a carbonate according to the following reactions. The carbonate exists in the form of carbonate ion (CO₃²⁻), which reacts with a metal cation to form a precipitate.



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In the mechanism, the hydration reaction of carbon dioxide is the rate-determining step and accelerated in the presence of a carbonic anhydrase. Further, the final product obtained after catalyzing the capture of carbon dioxide is ready to react with different metal cations, such as calcium ion (Ca²⁺), manganese ion (Mn²⁺), iron ion (Fe²⁺), etc., to form different carbonates, such as calcium carbonate (CaCO₃), manganese carbonate (MnCO₃), iron carbonate (FeCO₃), etc. These carbonates can be used in various industrial applications for different use purposes.

The presence of a carbonic anhydrase can accelerate the precipitation as well as the catalytic hydration of carbon dioxide. This is because the carbonic anhydrase catalyzes the hydration to promote the rate of forming carbonate ions, resulting in the faster precipitation of the carbonate.

Despite the catalytic function of carbonic anhydrase, the extraction of carbonic anhydrase from the nature for industrial use purposes has been limited due to the expense of enzyme purification and additional enzyme fixation. Bovine carbonic anhydrase extracted from bovine serum has been widely used, but its practical utilization is limited because it costs high as much as about three thousand dollars per gram. The techniques for extraction and purification of carbonic anhydrase from organisms have been developed incompletely, and the genetically recombinant carbonic anhydrase has been studied only for the biochemical research on enzymes. However, there have ever been yet made attempts neither to convert carbon dioxide to carbonates using a recombinant carbonic anhydrase which can be produced in large scale, nor to utilize a recombinant whole cell as a catalyst.

In an attempt to develop a technique for conversion of carbon dioxide to carbonates using a carbonic anhydrase with high efficiency at low cost, the inventors of the present invention have contrived a recombinant carbonic anhydrase available in practical use and a recombinant whole cell biocatalyst using transformant cells expressing the recombinant carbonic anhydrase in large scale. To complete the present invention, the inventors prepared a vector including a carbonic anhydrase gene and successfully expressed the vector in *Escherichia coli* in large scale. They also demonstrated that both the recombinant carbonic anhydrase produced from the vector and the whole cell biocatalyst expressing a carbonic anhydrase had high activity on the hydration of carbon dioxide, and the use of recombinant carbonic anhydrase contributed to effective conversion of carbon dioxide to carbonate.

DISCLOSURE

Technical Problem

It is an object of the present invention to provide a composition for capturing carbon dioxide that comprises at least one selected from the group consisting of: a whole cell of a transformant formed with a vector including a nucleic acid encoding a carbonic anhydrase; a cell lysate or its fraction of the whole cell; and a carbonic anhydrase isolated from the whole cell.

It is another object of the present invention to provide a method for capturing carbon dioxide using the composition for capturing carbon dioxide.

It is still another object of the present invention to provide a composition for converting carbon dioxide to a carbonate or a bicarbonate that comprises the CO₂ capturing composition and a metal cation.

It is still further another object of the present invention to provide a method for converting carbon dioxide to a carbonate or a bicarbonate using the composition.

Technical Solution

In one preferred embodiment of the present invention to achieve the above objects, the present invention is directed to a composition for capturing carbon dioxide that comprises at least one selected from the group consisting of: a whole cell of a transformant formed with a vector including a nucleic acid encoding a recombinant carbonic anhydrase; a cell lysate or its fraction of the whole cell; or a carbonic anhydrase isolated from the whole cell.

The term "carbonic anhydrase (CA)" as used herein refers to a Zn-containing metallic enzyme that catalyzes the hydration of carbon dioxide ($\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{HCO}_3^-$). The bovine carbonic anhydrase derived from bovine serum as a conventional carbonic anhydrase for industrial use has been limited in its practical utilization due to the difficulty of purification and high expense of production. However, the present invention provides a carbonic anhydrase expressed in large scale by genetic recombination and useful as a whole cell catalyst to capture carbon dioxide and prepare carbonates from carbon dioxide with ease at low cost, with high catalytic activity equivalent to that of the conventional enzyme from bovine serum.

The present invention features a carbonic anhydrase expressed by genetic recombination. The carbonic anhydrase may be derived from any organism as long as it has a catalytic function on the hydration of carbon dioxide. For example, the carbonic anhydrase of the present invention can be derived from prokaryotes or eukaryotes and, more specifically from, if not limited to, gram-positive bacteria, gram-negative bacteria, bacteria, fungi, yeasts, plants, animals, or human.

Preferably, the carbonic anhydrase of the present invention may be derived from *Synechocystis* PCC6803, *Escherichia coli*, or *Neisseria gonorrhoeae* and expressed by genetic recombination. The growth of *Neisseria gonorrhoeae* is accelerated the presence of carbon dioxide. The carbonic anhydrase from *Neisseria gonorrhoeae* exists as a monomer and has a high k_{cat}/K_M value approximating 46% with respect to human carbonic anhydrase II (HCA II) which is known to have the highest k_{cat}/K_M value among the existing carbonic anhydrases, so that it can be readily secreted from the periplasmic space or cell surface to desirably enhance the catalytic efficiency. This example is given only to exemplify the present invention and not intended to limit the scope of the present invention.

Preferably, the carbonic anhydrase of the present invention may be used in the form of at least one selected from the group consisting of: a whole cell of a transformant formed with a vector including a nucleic acid encoding a carbonic anhydrase; a cell lysate or its fraction of the whole cell; and a carbonic anhydrase isolated from the whole cell. The fraction may include a soluble fraction, an insoluble fraction, a cytoplasmic fraction, a periplasmic fraction, or a cell membrane fraction of the cell lysate.

Preferably, the transformant cell may be expressed in cytoplasm, periplasmic space, or cell surface.

For this, as a preferred example, the carbonic anhydrase from *Neisseria gonorrhoeae* may be a protein having an amino acid sequence of SEQ ID NO: 1, which is expressed in cytoplasm. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 1 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 2.

In the present invention, the carbonic anhydrase from *Neisseria gonorrhoeae* may also be a protein having an amino acid sequence of SEQ ID NO: 3 and produced in the periplasmic space, for the protein is formed by insertion of TorA as a signal sequence for inducing protein expression in *Escherichia coli* cytoplasm into the N-terminal domain of SEQ ID NO: 1. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 3 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 4.

In the present invention, the carbonic anhydrase from *Neisseria gonorrhoeae* may also be a protein having an amino acid sequence of SEQ ID NO: 5 and produced in the cell surface, for the protein is formed by insertion of an ice nucleation protein sequence as a surface anchoring motif for secretion in *Escherichia coli* cell surface into the N-terminal domain of SEQ ID NO: 1. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 5 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 6.

In the present invention, the carbonic anhydrase from *Synechocystis* may be a protein having an amino acid sequence of SEQ ID NO: 7, which protein is produced in the cytoplasm. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 7 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 8.

In the present invention, the carbonic anhydrase may also be a protein having an amino acid sequence of SEQ ID NO: 9 and produced in the periplasmic space, for the protein is formed by insertion of TorA as a signal sequence for protein expression in the *Escherichia coli* periplasmic space into the N-terminal domain of SEQ ID NO: 7. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 9 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 10.

In the present invention, the carbonic anhydrase from *Escherichia coli* may be a protein having an amino acid sequence of SEQ ID NO: 11 and produced in the cytoplasm. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 11 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 12.

In the present invention, the carbonic anhydrase may also be a protein having an amino acid sequence of SEQ ID NO: 13 and produced in the periplasmic space, for the protein is formed by insertion of TorA as a signal sequence for protein expression in the *Escherichia coli* periplasmic space into the N-terminal domain of SEQ ID NO: 11. The nucleic acid encoding the carbonic anhydrase having an amino acid sequence of SEQ ID NO: 13 may have, if not specifically limited to, a nucleic acid sequence of SEQ ID NO: 14.

The genetic recombination process for producing the biocatalyst of the present invention includes the following steps.

The first step is preparing a vector including a nucleic acid encoding a carbonic anhydrase.

The nucleic acid encoding a carbonic anhydrase may be preferably derived from, for example, *Neisseria gonorrhoeae*, *Synechocystis*, or *Escherichia coli*, and appropriately modified by a known method to be expressed in a desired region of the host cell, such as cytoplasm, periplasmic space, or cell surface. More specifically, the nucleic acid may encode an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, or SEQ ID NO: 13, and have a nucleic acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, or SEQ ID NO: 14.

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The vector for inserting the nucleic acid may be a recombinant vector in different forms of plasmid, virus, cosmid. The term "recombinant vector" as used herein refers to a double-stranded DNA fraction as a carrier with a foreign DNA fraction. The term "foreign DNA" as used herein refers to a DNA originated from a foreign species, or otherwise, a substantially modified form of the original DNA fraction. The foreign gene encodes a polypeptide with a specific nucleic acid to be transcribed. According to the object of the present invention, the foreign DNA means a nucleic acid encoding a carbonic anhydrase.

To enhance the expression level of a transformed gene in a host cell, the recombinant vector has the target gene operatively linked to a transcription/decoding/expression control sequence in the selected host cell. The recombinant vector is a gene construct which contains a necessary control factor operatively linked to express an inserted gene fraction in the target cell. The standard recombinant DNA technique is used to produce such a gene construct. The recombinant vector is not specifically limited as long as it can express a target gene and produce a target protein in all kinds of host cells such as prokaryotes or eukaryotes. The preferred recombinant vector has a promoter excellent in activity and expression ability and capable of large-scale expression of a foreign protein similar to the natural form. Preferably, the recombinant vector may include at least a promoter, an initiation codon, a gene for encoding a target protein, a termination codon, and a terminator. In addition, the recombinant vector may appropriately include a DNA for encoding a signal peptide, an enhancer sequence, non-coding regions for the 5' and 3' termini of a target gene, a selectable marker region, or a replicable unit.

In the specified examples of the present invention, a vector was prepared so that a nucleic acid encoding carbonic anhydrase derived from *Neisseria gonorrhoeae*, *Synechocystis*, or *Escherichia coli* was inserted into a vector pET22b(+) or pTrcHis, which vector had a cleavage map of FIG. 1.

The second step is preparing a transformant cell using the vector including a nucleic acid encoding a carbonic anhydrase.

The method for preparing a transformant by introducing a recombinant vector into a host cell may be a well-known method to introduce a nucleic acid into a cell, which method may include, if not specifically limited to, calcium phosphate- or calcium chloride/rubidium chloride-mediated transformation, electroporation, electroinjection, heat shock transformation, chemical transformation using chemicals such as PEG, gene gun transformation, retroviral infection, microinjection, DEAE-dextran transformation, cationic liposome transformation, or the like.

The host cell to be transformed with the recombinant vector of the present invention may be any prokaryote or eukaryote and required to have a high introduction efficiency of DNA and a high expression efficiency of the introduced DNA. The specific examples of the host cell may include known prokaryote or eukaryote cells, such as *Escherichia coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, Fungi, or yeasts; insect cells such as *Spodoptera Frugiperda* (SF9); animal cells, such as CHO, COS 1, COS 7, BSC 1, BSC 40, BMT 10, etc. Among these, *Escherichia coli* is preferred.

In the specified examples of the present invention, the vector, that is, an expression vector where a nucleic acid encoding a carbonic anhydrase was inserted in pET22b(+) or pTrcHis was introduced into *Escherichia coli* BL21 (DE3) through heat shock transformation at 42° C. for 2 minutes to prepare a transformant for large-scale production of carbonic anhydrase.

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The third step is culturing the transformant to induce expression of carbonic anhydrase and accumulate the produced carbonic anhydrase.

The cultivation of the transformant expressing the recombinant vector in a nutrient medium can produce and isolate a useful protein in large scale. The medium and culture conditions are properly determined as accepted in the related art depending on the type of the host cell. Such conditions as temperature, pH value of the medium, and culture time can be properly controlled to favor the growth of the cell and the large-scale production of the protein during the cultivation. IPTG (isopropyl- β -D-thiogalactopyranoside) is used as an expression-inducing factor to induce protein expression, and the induction time is controlled to maximize the yield of the protein.

In the specified examples of the present invention, the transformed *Escherichia coli* cells were cultured in a LB medium supplemented with ampicillin. When the absorbance (OD₆₀₀) of the culture sample at 600 nm reached 0.6 to 0.8, IPTG as a substance for inducing protein expression and ZnSO₄ for introducing zinc (Zn) were added to the culture sample, which were then cultured at 25° C. for more 25 hours. But, these culture conditions can be properly modified by those skilled in the art.

To investigate the expression of the carbonic anhydrase according to the above-described method, the transformant cell thus harvested is suspended with an aqueous buffer solution, crushed with an ultrasonic pulverizer and then partly separated into a soluble fraction and an insoluble fraction on a typical SDS-PAGE.

The transformant with the enzyme expressed is useful as a whole cell biocatalyst. More preferably, at least one selected from the group consisting of the transformant (whole cell), the lysate or its fraction of the transformant cell, and a carbonic anhydrase isolated from the transformant cell may be used as a biocatalyst.

In accordance with a preferred embodiment of the present invention, the recombinant carbonic anhydrase may be used as a whole cell biocatalyst produced in cytoplasm, periplasmic space, or cell surface without a process for separation and purification of carbonic anhydrase.

In accordance with another preferred embodiment of the present invention, the transformant cell expressing a carbonic anhydrase may be destroyed by different physical or chemical means, such as repetitive freeze-thawing, ultrasonic waves, mechanical destruction, or cell-degrading agents. The cell lysate containing the destroyed cells may be directly used as a biocatalyst.

In accordance with still another preferred embodiment of the present invention, the cell lysate containing the destroyed cells may be separated into a soluble fraction and an insoluble fraction, both of which are useful as a biocatalyst. Further, a cytoplasm fraction, a periplasmic fraction, or a cell membrane fraction may also be used as a biocatalyst.

In accordance with still another preferred embodiment of the present invention, the carbonic anhydrase produced in the transformant cell may be used as a biocatalyst after isolation and purification. The carbonic anhydrase thus produced can be isolated and purified by known biochemical isolation techniques after pulverization of the transformant cell. For example, the isolation and purification methods may include, if not specifically limited to, electrophoresis, centrifugal separation, gel filtration, precipitation, dialysis, chromatography (ion-exchange chromatography, affinity chromatography, immune-affinity chromatography, reverse HPLC, gel permeation HPLC, etc.), isoelectric focusing, or various modifications or combinations of these methods.

In a specified example of the present invention, to obtain a carbonic anhydrase derived from *Neisseria gonorrhoeae*, the transformant cells harvested were destroyed with an ultrasonic pulverizer, and the soluble fraction of the cell lysate was subjected to affinity chromatography using a column filled with a nickel resin to isolate and purify a desired carbonic anhydrase. The purified protein was removed of the salt (imidazole) remaining in the aqueous protein solution through dialysis using tris-sulfate (pH 7.6). The purification of the target carbonic anhydrase was investigated using SDS-PAGE and Western Blotting (See. FIG. 7).

In another specified example of the present invention, the purified enzyme was analyzed in regard to activity on hydration of carbon dioxide. Then, the whole cell and the soluble fraction obtained by destruction of the whole cell with an ultrasonic pulverizer were analyzed in regard to activity on hydration of carbon dioxide. The positive control was commercial bovine carbonic anhydrase extracted from bovine serum, and the negative control was bovine serum albumin inactive on the hydration of carbon dioxide. The results showed that all of the transformed whole cell, its soluble fraction, and the carbonic anhydrase isolated from the soluble fraction had such a high activity of capturing carbon dioxide as comparable to the positive control (See. FIG. 8).

In accordance with further another embodiment of the present invention, there is provided a method for capturing carbon dioxide using the composition for carbon dioxide.

More specifically, the present invention is directed to a method for capturing carbon dioxide that comprises: preparing the composition for capturing carbon dioxide; and feeding carbon dioxide into the composition for capturing carbon dioxide.

The preparation of the composition for capturing carbon dioxide may include: (1) preparing a vector including a nucleic acid encoding a carbonic anhydrase; (2) preparing a transformant cell formed with the vector; (3) culturing the transformant cell to induce expression of carbonic anhydrase and accumulate the carbonic anhydrase; and (4) preparing a composition including at least one selected from the group consisting of the transformant cell, a cell lysate or its fraction of the transformant cell, and the carbonic anhydrase isolated from the transformant cell.

The method of feeding carbon dioxide into the composition for capturing carbon dioxide may include, if not specifically limited to, feeding a source of carbon dioxide that contains a large amount of carbon dioxide and needs to be removed of carbon dioxide, such as in the form of waste water or flue gas.

After capturing carbon dioxide with the composition for capturing carbon dioxide according to the present invention, a source of metal cation is added to the composition to convert the captured carbon dioxide to a carbonate/bicarbonate precipitate, which is useful for industrial use purpose in various applications.

In accordance with still another embodiment of the present invention, there is provided a composition for converting carbon dioxide to a carbonate or a bicarbonate that comprises the composition for capturing carbon dioxide, and a metal cation.

According to the present invention, there is also provided a method for converting carbon dioxide to a carbonate or a bicarbonate using the composition.

Preferably, the present invention is directed to a method for converting carbon dioxide to a carbonate or a bicarbonate that includes: preparing the composition for capturing carbon dioxide; and feeding a metal cation and carbon dioxide into the composition for capturing carbon dioxide.

The term "carbonate" or "carbonate precipitate" as used herein refers to an inorganic component containing a carbonate group ($-\text{CO}_3$). This term may include both a mixture of carbonate and bicarbonate, and a species containing a carbonate ion alone. The term "bicarbonate" or "bicarbonate precipitate" as used herein refers to an inorganic component containing a bicarbonate group ($-\text{HCO}_3$). This term may include both a mixture of carbonate and bicarbonate, and a species containing a bicarbonate ion alone.

The source of metal ion that reacts with carbon dioxide to form a carbonate or a bicarbonate is not specifically limited as long as it contains a metal ion, and can be properly chosen according to its use. The preferred source of metal ion may react with the source of carbonate to produce a carbonate in a crystal form of calcite, aragonite or vaterite, or in an amorphous crystal form.

For example, the source of metal ion may be Na^+ , Ca^{2+} , Fe^{2+} , Mn^{2+} , Sr^{2+} , Ca^{2+} , Ba^{2+} , Zn^{2+} , or Pb^{2+} , or its nitrate, hydrochloride, hydrate or alkaline solution.

The carbonate precipitate prepared with the source of metal ion may include, if not specifically limited to, sodium carbonate, calcium carbonate, iron carbonate, manganese carbonate, strontium carbonate, barium carbonate, zinc carbonate, or lead carbonate.

In the step of feeding a metal cation and carbon dioxide into the composition for capturing carbon dioxide according to the present invention, the metal cation and the carbon dioxide may be supplied in sequence or simultaneously. For example, the sequential feeding is conducted in the order of metal cation and carbon dioxide, or in the order of carbon dioxide and metal cation; otherwise, the simultaneous feeding of the metal cation and the carbon dioxide is conducted.

In a specified example of the present invention, calcium ions were fed into the carbon dioxide captured with the purified carbonic anhydrase of the present invention, the soluble fraction, and the whole cell. Then, the production of calcium carbonate was investigated with an X-ray diffractometer (XRD) and a scanning electron microscope (SEM) (See. FIGS. 11 and 12).

The carbonate or bicarbonate produced by reaction between carbon dioxide and a metal cation is useful in the industrial applications. For example, the carbonate or bicarbonate may be used as an inorganic filler in a wide range of industrial applications, such as rubber, plastic, paper, paint, coating, adhesive, cosmetics, medicine and medical supplies, and so forth.

Calcium carbonate is one of the minerals present in most quantity in the nature. Particularly, the calcium carbonate precipitate is an inorganic powder with an adequate specific gravity that is insoluble in pure water and characterized by high whiteness and non-inflammability, so that it can be used as an inorganic filler in a wide range of industrial applications. When used as a raw material, such as a filler for rubber, plastic, or paint, or a pigment for paper making, the aragonite-type calcium carbonate precipitate, which is of a needle-like shape with a considerably high aspect ratio (the ratio of crystal length to size), can enhance strength and whiteness and make opacity controllable due to its complicated needle-like surface structure, so that it is useful as an alternative as a novel functional inorganic powder capable of providing mechanical and optical functions. In this manner, the present invention can provide usefulness in producing calcium carbonate.

Advantageous Effects

The present invention is economically beneficial in that carbon dioxide can be captured using a supernatant liquid of

the cell lysate or a transformant cell expressing carbonic anhydrase as a whole cell biocatalyst without a need of separately extracting the enzyme. Further, the final product is converted to a high value-added carbonate and thus can be utilized for various use purposes in the industrial applications, such as of paint, plastic, rubber, paper making, coating, adhesive, cosmetics, medicine and medical supplies, and so forth.

DESCRIPTION OF DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

FIG. 1 shows cleavage maps of expression vectors containing a carbonic anhydrase gene: (A) a vector for cytoplasmic expression; (B) a vector for periplasmic expression; and (C) a vector for cell surface expression.

FIG. 2 presents the results of (A) SDS-PAGE analysis and (B) Western Blot analysis for a whole cell fraction (T), a soluble fraction (S), and an insoluble fraction (IS), when a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in cytoplasm.

FIG. 3 presents the results of a Western Blot analysis for a soluble fraction (S), an insoluble fraction (IS), and a periplasmic fraction (PP), when a carbonic anhydrase from *Synechocystis* is expressed in cytoplasm (SCA) and periplasmic space (TSCA).

FIG. 4 presents the results of a Western Blot analysis for a whole cell fraction (T), a soluble fraction (S), an insoluble fraction (IS), and a periplasmic fraction (PP) when a carbonic anhydrase from *Escherichia coli* is expressed in cytoplasm (ECA) and periplasmic space (TECA).

FIG. 5 presents the results of a Western Blot analysis for a whole cell fraction (T), a cell lysate (CL), a soluble fraction (S), an insoluble fraction (IS), and a periplasmic fraction (PP), when a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in periplasmic space.

FIG. 6 presents the results of a Western Blot analysis for a cell lysate (CL), a soluble fraction (S), an insoluble fraction (IS), a cytoplasmic fraction (CP), and a cell membrane fraction (TM), when a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in cell surface.

FIG. 7 presents the results of (A) SDS-PAGE analysis and (B) Western Blot analysis for a whole cell (W), a soluble fraction (S) and a purified fraction (P) of a transformant, where a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in cytoplasm.

FIG. 8 presents the measurement results of the catalytic activity on hydration of carbon dioxide for a whole cell (W), a soluble fraction (S) and a purified fraction (NCA) of a transformant in which a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in cytoplasm. Here, BSA is bovine serum albumin as a negative control, and BCA is bovine carbonic anhydrase as a positive control.

FIG. 9 presents pictures showing the precipitate shape and dried calcium carbonate powder two minutes after the initial reaction for forming a calcium carbonate precipitate using a whole cell, a soluble fraction and a purified fraction of a transformant in which a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in cytoplasm, a positive control (BSA), and a negative control (BCA).

FIG. 10 presents pictures showing the dried calcium carbonate powder one minute after the initial reaction for forming a calcium carbonate precipitate using a whole cell in

which a carbonic anhydrase from *Neisseria gonorrhoeae* is expressed in cytoplasm or periplasmic space.

FIG. 11 shows the results of X-ray diffraction on each calcium carbonate powder of FIG. 9 to identify the crystal type.

FIG. 12 shows scanning electron microscope (SEM) pictures of each calcium carbonate powder of FIG. 9 to identify the crystal shape.

FIG. 13 is a mimetic diagram showing the process of capturing carbon dioxide and converting it to a carbonate using a recombinant whole cell biocatalyst expressing a recombinant carbonic anhydrase in cytoplasm, periplasmic space, or cell surface of a transformant cell.

MODE FOR INVENTION

Hereinafter, the present invention will be described in detail with reference to examples, which are given only to exemplify the present invention and not intended to limit the scope of the present invention.

Example 1

Preparation of Carbonic Anhydrase Expression Vector

1-1. Preparation of Vector for Cytoplasmic Expression

A carbonic anhydrase gene of *Neisseria gonorrhoeae* was amplified using two primers of a *Neisseria gonorrhoeae* genome DNA (i.e., forward primer: 5'-CATATGCACG-GCAATCACACC-3' (SEQ ID NO: 15), and backward primer: 5'-AAGCTTTTCAATACTACACGTGCATT-3' (SEQ ID NO: 16)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a pET-22b(+) vector using a NdeI/XhoI restriction enzyme to prepare a cytoplasmic expression vector for a carbonic anhydrase derived from *Neisseria gonorrhoeae*.

A carbonic anhydrase gene of *Synechocystis* was amplified using two primers of a *Synechocystis* genome DNA (i.e., forward primer: 5'-CATATGGCCGAAGTTTCAT-TGATATCC-3' (SEQ ID NO: 17), and backward primer: 5'-CAAGCTTACGGGAGCCTCGATAAATGCGC-3' (SEQ ID NO: 18)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a TorA-GFP-removed pTTG vector (Korean Patent Application No. 2005-0099758) using a NdeI/HindIII restriction enzyme to prepare a cytoplasmic expression vector for a carbonic anhydrase derived from *Synechocystis*.

A carbonic anhydrase gene of *Escherichia coli* was amplified using two primers of a *Escherichia coli* genome DNA (i.e., forward primer: 5'-CATATGAAAGAGATTATTGATG-GATTCC-3' (SEQ ID NO: 19), and backward primer: 5'-CAAGCTTCGCTGCGGTTCGGTTGGCGTAG-3' (SEQ ID NO: 20)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a TorA-GFP-removed pTTG vector using

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a NdeI/HindIII restriction enzyme to prepare a cytoplasmic expression vector for a carbonic anhydrase derived from *Escherichia coli*.

1-2. Preparation of Vector for Periplasmic Expression

A carbonic anhydrase gene of *Neisseria gonorrhoeae* was amplified using two primers of a *Neisseria gonorrhoeae* genome DNA (i.e., forward primer: 5'-CCATGGGACACG-GCAATCACACC-3' (SEQ ID NO: 21), and backward primer: 5'-AAGCTTTTCAATACTACACGTGCATT-3' (SEQ ID NO: 16)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a pET-22b(+) vector using a NdeI/XhoI restriction enzyme to prepare a periplasmic expression vector for a carbonic anhydrase derived from *Neisseria gonorrhoeae*.

A carbonic anhydrase gene of *Synechocystis* was amplified using two primers of a *Synechocystis* genome DNA (i.e., forward primer: 5'-CCATGGGAGCCGAAGTTTCAT-TGATATCC-3' (SEQ ID NO: 22), and backward primer: 5'-CAAGCTTACGGGAGCCTCGATAAATGCGC-3' (SEQ ID NO: 18)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a GFT-removed pTTG vector (Korean Patent Application No. 2005-0099758, filed on Oct. 21, 2005) using a NcoI/HindIII restriction enzyme to prepare a periplasmic expression vector for a carbonic anhydrase derived from *Synechocystis*.

A carbonic anhydrase gene of *Escherichia coli* was amplified using two primers of a *Escherichia coli* genome DNA (i.e., forward primer: 5'-CCATGGGAAAAGATTAT-TGATGGATTC-3' (SEQ ID NO: 23), and backward primer: 5'-CAAGCTTCGCTGCGGTTCGGTTGGCGTAG-3' (SEQ ID NO: 20)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a TorA-GFP-removed pTTG vector using a NcoI/HindIII restriction enzyme to prepare a periplasmic expression vector for a carbonic anhydrase derived from *Escherichia coli*.

1-3. Preparation of Vector for Cell Surface Expression

A carbonic anhydrase gene of *Neisseria gonorrhoeae* was amplified using two primers of a *Neisseria gonorrhoeae* genome DNA (i.e., forward primer: 5'-AGATCTCACG-GCAATCACACCCATTGG-3' (SEQ ID NO: 24), and backward primer: 5'-AAGCTTTTCAGTGGTGGTGGTGGTGGTGGTGG-3' (SEQ ID NO: 25)), a Taq DNA polymerase, a dNTP mixed solution, and a PCR buffer by carrying out 30 cycles of PCR (30-sec denaturation at 95° C., 30-sec coupling at 55° C. and one-minute polymerization reaction at 72° C.) and then cooling down to 4° C. The carbonic anhydrase gene thus amplified was introduced into a pINPNC-OPH vector (Li L, Kang D G, Cha H J. 2004, Biotechnol Bioeng 85:214-221) using a BglII/HindIII restriction enzyme to prepare a cell surface expression vector for a carbonic anhydrase derived from *Neisseria gonorrhoeae*.

Example 2

Preparation of Transformant Including Cytoplasmic, Periplasmic, or Cell Surface Expression Vector

Each of the cytoplasmic expression vector, the periplasmic expression vector, and the cell surface expression vector pro-

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duced in Examples 1-1, 1-2, and 1-3, respectively, was introduced into *Escherichia coli* BL21(DE3) by heat shock transformation at 42° C. for 2 minutes, to prepare a transformant including each vector and expressing a recombinant carbonic anhydrase. Each of the vector-inserted transformants was sorted out in an LB medium supplemented with ampicillin.

Example 3

Protein Expression and Preparation of Whole Cell Biocatalyst Using Each Transformant

Each transformant prepared in Example 2 was cultured in a general LB medium (37° C.) supplemented with 50 µg/mL of ampicillin. When the absorbance (OD₆₀₀) of the culture sample reached 0.6 to 0.8, an expression-inducing factor, IPTG (isopropyl-D-thiogalactopyranoside), was added to induce protein expression. The cultured cell was then cultured at 25° C. for 20 more hours and then subjected to centrifugal separation at 4,000 rpm for 10 minutes. The culture sample was removed of the supernatant liquid to harvest cells, which were suspended in a solution for cell lysis (50 mM sodium phosphate buffer, 300 mM NaCl, pH 8) and destroyed with an ultrasonic pulverizer.

Example 3-1

Investigation of Cytoplasmic Expression

The destroyed cell including a cytoplasmic expression vector for a carbonic anhydrase from *Neisseria gonorrhoeae* was divided into a whole cell fraction (T) and its soluble fraction (S) and insoluble fraction (IS) and then subjected to SDS-PAGE and Western Blot analyses. As shown in FIG. 2, the analysis results revealed that the recombinant carbonic anhydrase was highly expressed in *Escherichia coli* with a molecular weight of about 25 kDa, which approximated the theoretical molecular weight of the carbonic anhydrase, 25.3 kDa. It was also revealed that the expressed protein was mostly folded into a native structure and expressed as the soluble fraction (lane S) having an activity.

The destroyed cell (SCA) including a cytoplasmic expression vector of a carbonic anhydrase from *Synechocystis* was divided into a soluble fraction (S) and an insoluble fraction (IS) and then subjected to Western Blot analysis. As shown in FIG. 3, the recombinant carbonic anhydrase had a molecular weight of about 34 kDa approximating the theoretical molecular weight of the carbonic anhydrase. The protein was mostly expressed as the soluble fraction (lane S).

The destroyed cell (ECA) including a cytoplasmic expression vector of a carbonic anhydrase from *Escherichia coli* was divided into a whole cell fraction (T), a soluble fraction (S), and an insoluble fraction (IS) and then subjected to Western Blot analysis. As shown in FIG. 4, the recombinant carbonic anhydrase had a molecular weight of about 23 kDa approximating the theoretical molecular weight of the carbonic anhydrase. But, the protein was mostly expressed as the insoluble fraction (lane IS).

Example 3-2

Investigation of Periplasmic Expression

The destroyed cell including a periplasmic expression vector for a carbonic anhydrase from *Neisseria gonorrhoeae* was divided into a whole cell fraction (T), a cell lysate (CL), a soluble fraction (S), an insoluble fraction (IS), and a periplas-

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mic fraction (PP) and then subjected to Western Blot analysis. To obtain a periplasmic fraction, the cell lysate was centrifugally separated at 4,000 rpm for 10 minutes, removed of the supernatant liquid, suspended with a TEX buffer (50 mM tris, 3 mM EDTA, 0.1% Triton X-100, pH 8.0) and then stirred for one hour. Another centrifugal separation at 4,000 rpm for 10 more minutes turned the supernatant liquid into the periplasmic fraction (PP).

As shown in FIG. 5, the recombinant carbonic anhydrase thus produced had a molecular weight which approximated both the theoretical molecular weights of a carbonic anhydrase expressed with a signal sequence (28 kDa) and a carbonic anhydrase expressed without a signal sequence (25.3 kDa). Further, a band detection in the periplasmic fraction showed that the recombinant carbonic anhydrase thus produced was successfully secreted into the periplasmic space (lane PP).

The destroyed cell (TSCA) including a periplasmic expression vector for a carbonic anhydrase from *Synechocystis* was divided into a soluble fraction (S), an insoluble fraction (IS), and a periplasmic fraction (PP) and then subjected to Western Blot analysis. As shown in FIG. 3, the recombinant carbonic anhydrase thus produced was not efficiently secreted into the periplasmic space.

The destroyed cell (TECA) including a periplasmic expression vector for a carbonic anhydrase from *Escherichia coli* was divided into a whole cell fraction (T), a soluble fraction (S), an insoluble fraction (IS), and a periplasmic fraction (PP) and then subjected to Western Blot analysis. As shown in FIG. 4, the recombinant carbonic anhydrase thus produced was secreted in large quantity into the periplasmic space (lane PP).

Example 3-3

Investigation of Cell Surface Expression

The destroyed cell including a cell surface expression vector for a carbonic anhydrase derived *Neisseria gonorrhoeae* was divided into a cell lysate (CL), a soluble fraction (S), an insoluble fraction (IS), and a cytoplasmic fraction (CP), and further into a cytoplasmic fraction and a cell membrane fraction in order to investigate the expression in a cell membrane fraction (TM). The process of preparing the cytoplasmic fraction and the cell membrane fraction was conducted as follows. The cultured cell was harvested after centrifugal separation, washed with PBS (130 mM NaCl, 2.5 mM KCl, 10 mM Na₂HPO₄, 1.7 mM KH₂PO₄) and then subjected to centrifugal separation at 9,000 rpm for 30 minutes.

With the supernatant liquid discarded, a solution containing 10 µg/ml of 1 mM EDTA in PBS was added to the cultured cell, which was stood for 2-hour reaction and then crushed with an ultrasonic pulverizer. The cell lysate thus obtained was centrifugally separated with an ultracentrifuge at 39,000 rpm for one hour to give a whole cell membrane fraction (TM) as the sediment and a cytoplasmic fraction (CP) as the supernatant.

As shown in FIG. 6, a protein band detected turned out to have a molecular weight of about 55 kDa, which was closely equivalent to the sum of the theoretical molecular weights of INPNC (25 kDa) and carbonic anhydrase (25.3 kDa) as expressed together, demonstrating the expression of the recombinant carbonic anhydrase in the cell surface (lane TM). In fact, it has been reported that the protein expressed

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together with INPNC appeared to have a greater molecular weight than theoretically calculated (J. Biotechnol., vol. 118 (4), pp. 339-470).

Example 4

Purification of Carbonic Anhydrase Expressed in Cytoplasm

The soluble fraction of the whole cell biocatalyst from *Escherichia coli* that was prepared in Example 3-1 and proved out to express carbonic anhydrase in cytoplasm was subjected to nickel column chromatography to isolate and purify a protein. More specifically, a soluble fraction of protein was poured into a column filled with a nickel resin, so the column absorbed the protein. Then a wash buffer (50 mM sodium phosphate buffer, 300 mM NaCl, 40 mM imidazole, pH 8.0) was used to wash away the protein not bound to the column. The protein was eluted from the column with a 50 mM sodium phosphate buffer, 300 mM NaCl, and 250 mM imidazole (pH 8.0). The purified solution was then dialyzed using 100 mM tris-sulfate (pH 7.6) to eliminate the salts.

FIG. 7 shows the results of (A) SDS-PAGE analysis and (B) Western Blot analysis for the purified protein (P), the whole cell fraction (W), and the soluble fraction (S). As can be seen from FIG. 7, the isolation and purification yielded the recombinant carbonic anhydrase with a high purity of 99% or greater (lane P). Further, the produced amount of the carbonic anhydrase protein was 10.6.2 mg/L, which was about 70% of the total amount of the protein (1.51.7 mg/L), and the carbonic anhydrase protein was purified to give 66.7 mg/l of pure carbonic anhydrase with the purification yield of 62.8%.

Example 5

Measurement of Activity on Hydration of Carbon Dioxide Using Isolated/Purified Carbonic Anhydrase and Recombinant Whole Cell Biocatalyst

The activity on the hydration of carbon dioxide was measured in the case of using the protein (NCA) purified in Example 4, or the soluble fraction (S) and the whole cell (W) of the *Escherichia coli* whole cell biocatalyst of Example 3 where a carbonic anhydrase was expressed in cytoplasm. The positive control was commercial bovine carbonic anhydrase (BCA) extracted from bovine serum, and the negative control was bovine serum albumin (BSA) inactive on the hydration of carbon dioxide. For the measurement of activity, each fraction with a 20 mM tris-sulfate buffer (pH 8.3) and a CO₂-saturated H₂O solution was investigated in regard to the required time to reduce the pH value from 8.0 to 7.0. The faster drop of the pH value indicates the higher CO₂ capturing activity.

As shown in FIG. 8, the purified carbonic anhydrase (NCA; ~2,200 U/mg) had the CO₂ capturing activity that was about 71% of the activity of the BCA (3,090 U/mg). Further, the non-purified fractions, that is, the solution fraction (S; ~920 U/mg) and the whole cell (W; ~730 U/mg) also had a high activity on the hydration of carbon dioxide.

Example 6

Precipitation of Calcium Carbonate Using Calcium Ion

Using a 200 mM tris-sulfate buffer (pH 10.5) and a 100 mM CaCl₂ solution as a source of calcium ions, the precipi-

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tation of calcium carbonate was induced in the negative control (BSA) and the positive control (BCA) of Example 5, three samples of cytoplasmic expression cells (i.e., purified carbonic anhydrase, solution fraction, and whole cell), and the periplasmic expression whole cell. More specifically, the buffer and the CaCl₂ solution (each 20 mL) were mixed with each sample. Under agitation, CO₂ gas was injected into the mixture at a defined flow rate to investigate the precipitation behavior.

FIG. 9 shows a quantitative comparison of precipitate powders prepared by filtering the precipitates produced 2 minutes after the supply of CO₂ gas through a 0.2 μm membrane filter and drying at 80° C. for about 30 minutes. Due to the function of the carbonic anhydrase, the precipitation as well as the catalyzation on hydration of carbon dioxide was accelerated in the BCA, the purified fraction, the solution fraction, and the cytoplasmic expression whole cell other than the BSA. The precipitate obtained from each sample was dried to yield calcium carbonate powder in large quantity.

Using a 200 mM tris-Cl buffer (pH 10.8) and a 500 mM CaCl₂ solution as a source of calcium ions, the precipitation of calcium carbonate was induced in the three samples of whole cells (i.e., the negative control, the cytoplasmic expression whole cell, and the periplasmic expression whole cell). The negative control whole cell was *Escherichia coli* cells containing a pET22(+) vector alone. More specifically, the buffer (23 mL) and the CaCl₂ solution (6 mL) were mixed with each whole cell. Under agitation, CO₂ gas was injected into the mixture at a defined flow rate to investigate the precipitation behavior.

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FIG. 10 shows a quantitative comparison of precipitate powders prepared by filtering the precipitates produced one minute after the supply of CO₂ gas through a 0.2 μm membrane filter and drying at 80° C. for about 30 minutes. As a result, the periplasmic expression whole cell yielded calcium carbonate powder about 6.3 times as much as the negative control and about 2.6 times as much as the cytoplasmic expression whole cell. Accordingly, the periplasmic expression, which reduces inhibition of material transfer by the cell membranes, can be an effective means for using the whole cell as a biocatalyst.

Example 7

Identification of Calcium Carbonate

Each powder obtained in Example 6 was identified as calcium carbonate crystals according to X-ray diffraction and SEM analyses. According to the X-ray diffraction peak patterns as shown in FIG. 11, the precipitate was calcium carbonate crystals consisting of calcite and vaterite together. The carbonic anhydrase of the positive control (BCA), the purified carbonic anhydrase, the soluble fraction, and the whole cell accelerated the transition of vaterite into calcite as well as precipitation. The negative control (BSA) having no enzymatic activity appeared to have little transition of vaterite into calcite due to retarded precipitation. As shown in FIG. 12, the precipitate was identified as calcium carbonate crystals with co-existence of parallelepiped calcite and spherical vaterite according to SEM analysis. Also, the use of the carbonic anhydrase accelerated the transition of vaterite into calcite as well as precipitation.

SEQUENCE LISTING

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Pro Ala Ile Lys Val Asn Tyr Lys Pro Ser Met Val Asp Val Glu Asn
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Asn Gly His Thr Ile Gln Val Asn Tyr Pro Glu Gly Gly Asn Thr Leu
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 85             90             95

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atgcacggca atcacacca ttggggctat accggacacg actctccga aagctggggc    60
aatctgtcag aagaattccg ttgtgtctcc accggcaaaa accaatctcc ggtaaacatt    120
accgaaacgg tttccggcaa actgcccggc atcaaagtca attacaaacc gagtatgggt    180
gacgtggaaa acaacggcca caccattcag gtcaattatc ccgaaggcgg caataccctg    240
accgtgaacg gccgcaccta taccctgaaa cagttccact tccacgtgcc gagcgaaaac    300
caaatcaaag gccgcacttt ccgatggaa gctcacttcg tccacttaga cgaaaacaaa    360
cagcctttag tattagccgt gctgtatgaa gccggcaaaa ccaacggccg cctgtcttcc    420
atctggaacg tcattgccgat gaccgcagga aaagtgaac tcaaccaacc gttcgacgca    480
tccaccctac tgccgaaaac gttgaaatac taccgctttg ccggttcgct gaccacgccc    540
ccgtgcacag agggcgtatc atggttggtg ttgaaaactt atgaccacat cgaccaagcg    600
caagcggaag aattcacccg cgccgtcggg tcggaaaaca accgccccgt acagcctctg    660
aatgcacgtg tagttattga aaagcttgcg gccgcactcg agcaccacca ccaccaccac    720
tga                                                                    723
  
```

<210> SEQ ID NO 3
 <211> LENGTH: 284
 <212> TYPE: PRT
 <213> ORGANISM: Neisseria gonorrhoeae
 <220> FEATURE:
 <221> NAME/KEY: PEPTIDE
 <222> LOCATION: (1)..(284)
 <223> OTHER INFORMATION: carbonic anhydrase (periplasmic expression)

<400> SEQUENCE: 3

Met Asn Asn Asn Asp Leu Phe Gln Ala Ser Arg Arg Arg Phe Leu Ala
 1 5 10 15
 Gln Leu Gly Gly Leu Thr Val Ala Gly Met Leu Gly Pro Ser Leu Leu
 20 25 30
 Thr Pro Arg Arg Ala Thr Ala Ala Gln Ala Ala Met Gly His Gly Asn
 35 40 45

-continued

His Thr His Trp Gly Tyr Thr Gly His Asp Ser Pro Glu Ser Trp Gly
 50 55 60
 Asn Leu Ser Glu Glu Phe Arg Leu Cys Ser Thr Gly Lys Asn Gln Ser
 65 70 75 80
 Pro Val Asn Ile Thr Glu Thr Val Ser Gly Lys Leu Pro Ala Ile Lys
 85 90 95
 Val Asn Tyr Lys Pro Ser Met Val Asp Val Glu Asn Asn Gly His Thr
 100 105 110
 Ile Gln Val Asn Tyr Pro Glu Gly Gly Asn Thr Leu Thr Val Asn Gly
 115 120 125
 Arg Thr Tyr Thr Leu Lys Gln Phe His Phe His Val Pro Ser Glu Asn
 130 135 140
 Gln Ile Lys Gly Arg Thr Phe Pro Met Glu Ala His Phe Val His Leu
 145 150 155 160
 Asp Glu Asn Lys Gln Pro Leu Val Leu Ala Val Leu Tyr Glu Ala Gly
 165 170 175
 Lys Thr Asn Gly Arg Leu Ser Ser Ile Trp Asn Val Met Pro Met Thr
 180 185 190
 Ala Gly Lys Val Lys Leu Asn Gln Pro Phe Asp Ala Ser Thr Leu Leu
 195 200 205
 Pro Lys Arg Leu Lys Tyr Tyr Arg Phe Ala Gly Ser Leu Thr Thr Pro
 210 215 220
 Pro Cys Thr Glu Gly Val Ser Trp Leu Val Leu Lys Thr Tyr Asp His
 225 230 235 240
 Ile Asp Gln Ala Gln Ala Glu Lys Phe Thr Arg Ala Val Gly Ser Glu
 245 250 255
 Asn Asn Arg Pro Val Gln Pro Leu Asn Ala Arg Val Val Ile Glu Lys
 260 265 270
 Leu Ala Ala Ala Leu Glu His His His His His His
 275 280

<210> SEQ ID NO 4
 <211> LENGTH: 855
 <212> TYPE: DNA
 <213> ORGANISM: Neisseria gonorrhoeae
 <220> FEATURE:
 <221> NAME/KEY: gene
 <222> LOCATION: (1)..(855)
 <223> OTHER INFORMATION: carbonic anhydrase (periplasmic expression)

<400> SEQUENCE: 4

atgaacaata acgatctctt tcaggcatca cgtcggcggtt ttctggcaca actcggcggc	60
ttaaccgtcg ccgggatgct ggggccgtca ttgttaacgc cgcgacgtgc gactgcggcg	120
caagcggccca tgggacacgg caatcacacc cattggggct ataccggaca cgactctccc	180
gaaagctggg gcaatctgtc agaagaattc cgtttgtgtc ccaccggcaa aaaccaatct	240
ccggtaaaca ttaccgaaac cgtttcgggc aaactgccc ccatcaaagt caattacaaa	300
ccgagtatgg ttgacgtgga aaacaacggc cacaccattc aggtcaatta tccgaaggc	360
ggcaataccc tgaccgtgaa cgcccgccacc tataccctga aacagttcca cttcacgtg	420
ccgagcgaaa accaaatcaa aggcgcact ttcccgatgg aagctcactt cgtccactta	480
gacgaaaaca aacagccttt agtattagcc gtgtgtatg aagccggcaa aaccaacggc	540
cgctgtctt ccatctggaa cgtcatgcc atgaccgcag gaaaagtga actcaaccaa	600
cggttcgacg catccacct actgccgaaa cggttgaaat actaccgctt tgccggttcg	660

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ctgaccacgc cgccgtgcac agagggcgta tcatggttgg tgttgaaaac ttatgaccac 720
atcgaccaag cgcaagcgga aaaattcacc cgcgccgtcg gttcggaaaa caaccgcccc 780
gtacagcctc tgaatgcacg tgtagttatt gaaaagcttg cggccgcact cgagcaccac 840
caccaccacc actga 855

```

```

<210> SEQ ID NO 5
<211> LENGTH: 462
<212> TYPE: PRT
<213> ORGANISM: Neisseria gonorrhoeae
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(462)
<223> OTHER INFORMATION: carbonic anhydrase (cell surface expression)

```

```

<400> SEQUENCE: 5

```

```

Met Ala Leu Asp Lys Ala Leu Val Leu Arg Thr Cys Ala Asn Asn Met
 1             5             10             15

Ala Asp His Cys Gly Leu Ile Trp Pro Ala Ser Gly Thr Val Glu Ser
 20             25             30

Arg Tyr Trp Gln Ser Thr Arg Arg His Glu Asn Gly Leu Val Gly Leu
 35             40             45

Leu Trp Gly Ala Gly Thr Ser Ala Phe Leu Ser Val His Ala Asp Ala
 50             55             60

Arg Trp Ile Val Cys Glu Val Ala Val Ala Asp Ile Ile Ser Leu Glu
 65             70             75             80

Glu Pro Gly Met Val Lys Phe Pro Arg Ala Glu Val Val His Val Gly
 85             90             95

Asp Arg Ile Ser Ala Ser His Phe Ile Ser Ala Arg Gln Ala Asp Pro
100             105             110

Ala Ser Thr Ser Thr Ser Thr Ser Thr Ser Thr Leu Thr Pro Met Pro
115             120             125

Thr Ala Ile Pro Thr Pro Met Pro Ala Val Ala Ser Val Thr Leu Pro
130             135             140

Val Ala Glu Gln Ala Arg His Glu Val Phe Asp Val Ala Ser Val Ser
145             150             155             160

Ala Ala Ala Ala Pro Val Asn Thr Leu Pro Val Thr Thr Pro Gln Asn
165             170             175

Leu Gln Thr Arg Ser Arg Leu Trp Asp Gly Lys Arg Tyr Arg Gln Leu
180             185             190

Val Ala Arg Thr Gly Glu Asn Gly Val Glu Ala Asp Ile Pro Tyr Tyr
195             200             205

Val Asn Glu Asp Asp Asp Ile Val Asp Lys Pro Asp Glu Asp Asp Asp
210             215             220

Trp Ile Glu Val His Gly Asn His Thr His Trp Gly Tyr Thr Gly His
225             230             235             240

Asp Ser Pro Glu Ser Trp Gly Asn Leu Ser Glu Glu Phe Arg Leu Cys
245             250             255

Ser Thr Gly Lys Asn Gln Ser Pro Val Asn Ile Thr Glu Thr Val Ser
260             265             270

Gly Lys Leu Pro Ala Ile Lys Val Asn Tyr Lys Pro Ser Met Val Asp
275             280             285

Val Glu Asn Asn Gly His Thr Ile Gln Val Asn Tyr Pro Glu Gly Gly
290             295             300

```

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Asn Thr Leu Thr Val	Asn Gly Arg Thr Tyr Thr	Leu Lys Gln Phe His
305	310	315 320
Phe His Val Pro Ser	Glu Asn Gln Ile Lys Gly Arg Thr	Phe Pro Met
	325	330 335
Glu Ala His Phe Val	His Leu Asp Glu Asn Lys Gln	Pro Leu Val Leu
	340	345 350
Ala Val Leu Tyr Glu	Ala Gly Lys Thr Asn Gly Arg	Leu Ser Ser Ile
	355	360 365
Trp Asn Val Met Pro	Met Thr Ala Gly Lys Val	Lys Leu Asn Gln Pro
	370	375 380
Phe Asp Ala Ser Thr	Leu Leu Pro Lys Arg Leu Lys Tyr Tyr	Arg Phe
	385	390 395 400
Ala Gly Ser Leu Thr	Thr Pro Pro Cys Thr Glu Gly Val	Ser Trp Leu
	405	410 415
Val Leu Lys Thr Tyr	Asp His Ile Asp Gln Ala Gln	Ala Glu Lys Phe
	420	425 430
Thr Arg Ala Val Gly	Ser Glu Asn Asn Arg Pro	Val Gln Pro Leu Asn
	435	440 445
Ala Arg Val Val Ile	Glu Leu Glu His His His	His His His
	450	455 460

<210> SEQ ID NO 6
 <211> LENGTH: 1389
 <212> TYPE: DNA
 <213> ORGANISM: Neisseria gonorrhoeae
 <220> FEATURE:
 <221> NAME/KEY: gene
 <222> LOCATION: (1)..(1389)
 <223> OTHER INFORMATION: carbonic anhydrase (cell surface expression)

<400> SEQUENCE: 6

atggctctcg acaaggcgtt ggtgctgcgt acctgtgcaa ataacatggc cgatcactgc	60
ggccttatat ggcccgcgtc cggcacggtg gaatccagat actggcagtc aaccaggcgg	120
catgagaatg gtctggtcgg tttactgtgg ggcgctggaa ccagcgcttt tctaagcgtg	180
catgccgatg ctcgatggat tgtctgtgaa gttgccgttg cagacatcat cagtctggaa	240
gagccgggaa tggteaagtt tccgcgggcc gaggtgggtc atgtcgcgca caggatcagc	300
gcgtcacact tcatttcggc acgtcaggcc gacctgcgt caacgtcaac gtcaacgtca	360
acgtcaacgt taacgccaat gcctacggcc ataccacgc ccatgcctgc ggtagcaagt	420
gtcacgttac cggtgggcga acaggcccgt catgaagtg tcatgtcgc gtcggtcagc	480
gcggtgcgcg cccagtaaa caccctgccg gtgacgacgc cgcagaattt gcagaccaga	540
tcaagactct gggacgggaa gaggtacagg caactggctg ccagaacggg tgagaacggt	600
gttgaggcgc acataccgta ttactgtgaa gaagatgacg atattgtcga taaaccgcac	660
gaggacgatg actggataga ggtacacggc aatcacaccc attggggcta taccggacac	720
gactctcccg aaagctgggg caatctgtca gaagaattcc gtttgtgctc caccggcaaa	780
aaccaatctc cggtaaacat taccgaaacc gtttcgggca aactgcccgc catcaaagtc	840
aattacaaac cgagtatggt tgacgtggaa aacaacggcc acaccattca ggtcaattat	900
cccgaaggcg gcaataccct gaccgtgaac ggccgcacct ataccctgaa acagtccac	960
ttccacgtgc cgagcgaaaa ccaaatcaaa ggccgcacct tcccgatgga agctcacttc	1020
gtccacttag acgaaaaaaa acagccttta gtattagccg tgctgtatga agccggcaaa	1080
accaacggcc gcctgtcttc catctggaac gtcacgcca tgaccgcagg aaaagtga	1140

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ctcaaccaac cgttcgaagc atccacccta ctgccgaaac ggttgaaata ctaccgcttt 1200
gccggttcgc tgaccacgcc gccgtgcaca gagggcgat catggttggt gttgaaaact 1260
tatgaccaca tcgaccaagc gcaagcggaa aaattcaccg gcgccgctcg ttcggaaaac 1320
aaccgccccg tacagcctct gaatgcacgt gtagttattg aactcgagca ccaccaccac 1380
caccactga 1389

```

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<210> SEQ ID NO 7
<211> LENGTH: 324
<212> TYPE: PRT
<213> ORGANISM: Synechocystis PCC6803
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(324)
<223> OTHER INFORMATION: carbonic anhydrase (cytoplasmic expression)

```

```

<400> SEQUENCE: 7

```

```

Met Ala Glu Val Ser Leu Ile Ser Gln Thr Asn Cys Pro Ala Val Leu
 1             5             10            15
Asp Pro Pro Pro Gln Val Phe Asn Asp Thr Met Glu Gly Trp Asn Ser
      20             25             30
Ile Asn Gly Asp Arg Met Gln Arg Leu Ile Glu Gly Leu Gln Lys Phe
      35             40             45
Arg Glu Gly Tyr Phe Ser Ser His Arg Asp Leu Phe Glu Gln Leu Ser
      50             55             60
His Gly Gln His Pro Arg Ile Leu Phe Ile Cys Cys Ser Asp Ser Arg
      65             70             75             80
Val Asp Pro Asn Leu Ile Thr Gln Ser Glu Val Gly Asp Leu Phe Val
      85             90             95
Ile Arg Asn Ala Gly Asn Ile Ile Pro Pro Tyr Gly Ala Ala Asn Gly
      100            105            110
Gly Glu Gly Ala Ala Met Glu Tyr Ala Leu Val Ala Leu Glu Ile Asn
      115            120            125
Gln Ile Ile Val Cys Gly His Ser His Cys Gly Ala Met Lys Gly Leu
      130            135            140
Leu Lys Leu Asn Ser Leu Gln Glu Lys Leu Pro Leu Val Tyr Asp Trp
      145            150            155            160
Leu Lys His Thr Glu Ala Thr Arg Arg Leu Val Leu Asp Asn Tyr Ser
      165            170            175
His Leu Glu Gly Glu Asp Leu Ile Glu Val Ala Val Ala Glu Asn Ile
      180            185            190
Leu Thr Gln Leu Lys Asn Leu Gln Thr Tyr Pro Ala Ile His Ser Arg
      195            200            205
Leu His Arg Gly Asp Leu Ser Leu His Gly Trp Ile Tyr Arg Ile Glu
      210            215            220
Glu Gly Glu Val Leu Ala Tyr Asp Gly Val Leu His Asp Phe Val Ala
      225            230            235            240
Pro Gln Ser Arg Ile Asn Ala Leu Glu Pro Glu Asp Glu Tyr Ala Pro
      245            250            255
His Pro Asn Ser Pro Leu Ile Ser Tyr Asp Ala Phe Lys Val Pro Gly
      260            265            270
Lys Glu Arg Pro Gly Arg Glu Lys Ala Thr Glu Ser Pro Ala Pro Gln
      275            280            285
Leu Ser Pro Leu Pro Gly Phe Gly His Leu Pro Arg Glu Gln Ala Glu
      290            295            300

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Arg Ile Tyr Arg Gly Ser Arg Lys Leu Ala Ala Ala Leu Glu His His
305 310 315 320

His His His His

<210> SEQ ID NO 8
<211> LENGTH: 975
<212> TYPE: DNA
<213> ORGANISM: Synechocystis PCC6803
<220> FEATURE:
<221> NAME/KEY: gene
<222> LOCATION: (1)..(975)
<223> OTHER INFORMATION: carbonic anhydrase (cytoplasmic expression)

<400> SEQUENCE: 8

```
atggccgaag tttcattgat atcccagaca aattgccccg ctgtcctaga cccccgcgcg      60
caagtcttca atgatacaat ggaaggctgg aattccatca acggcgatcg tatgcaaaga      120
ctcatcgagg gactacaaaa atttcagaaa gggtatttct cttcccacgg tgacctcttt      180
gagcaacttt ctcacggcca acatccccgc attctcttca tctgttggtc cgattcccgg      240
gtggacccca atttaatcac ccaatcggaa gtgggcgacc tgtttggtat tcgcaacgct      300
ggcaatatta ttcccccta tggagcagcc aacggtgggg aaggggcagc catggaatat      360
gccctagtgg cgctggaaat taatcagatc atcgtctgtg gccattccca ctgcggagcc      420
atgaaaggtc tgctcaaact caactctctc caggaaaaac ttctctgggt gtacgattgg      480
ctcaaacata cggaagccac ccgccgtcta gttctagaca attacagcca tctggaaggg      540
gaagatttga ttgaagtgc tgtggcagaa aatattctca cccaactcaa aaacctccag      600
acctatcccg ccattccatt ccggttacat cggggagacc tttccctcca cggttggtt      660
tatcgcatcg aagaggttga agtactggcc tacgacgggt tactccatga ttttgctgcc      720
ccccaaagtc gcataaatgc cctggagcgg gaggatgagt acgctcccca tcccaactca      780
cccctgattt cctacgatgc gtttaagggt cccggcaagg aacgtcctgg tctgtgagaaa      840
gcaacagaat cccagctcc ccaactgtct cctttacctg gttttggcca tttgccccagg      900
gaacaggcgg agcgcattta tcgaggetcc cgtaagcttg cggccgcact cgagcaccac      960
caccaccacc actga                                          975
```

<210> SEQ ID NO 9
<211> LENGTH: 368
<212> TYPE: PRT
<213> ORGANISM: Synechocystis PCC6803
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(368)
<223> OTHER INFORMATION: carbonic anhydrase (periplasmic expression)

<400> SEQUENCE: 9

```
Met Asn Asn Asn Asp Leu Phe Gln Ala Ser Arg Arg Arg Phe Leu Ala
  1           5           10          15

Gln Leu Gly Gly Leu Thr Val Ala Gly Met Leu Gly Pro Ser Leu Leu
  20          25          30

Thr Pro Arg Arg Ala Thr Ala Ala Gln Ala Ala Met Gly Ala Glu Val
  35          40          45

Ser Leu Ile Ser Gln Thr Asn Cys Pro Ala Val Leu Asp Pro Pro Pro
  50          55          60

Gln Val Phe Asn Asp Thr Met Glu Gly Trp Asn Ser Ile Asn Gly Asp
  65          70          75          80
```

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Arg Met Gln Arg Leu Ile Glu Gly Leu Gln Lys Phe Arg Glu Gly Tyr
 85 90 95
 Phe Ser Ser His Arg Asp Leu Phe Glu Gln Leu Ser His Gly Gln His
 100 105 110
 Pro Arg Ile Leu Phe Ile Cys Cys Ser Asp Ser Arg Val Asp Pro Asn
 115 120 125
 Leu Ile Thr Gln Ser Glu Val Gly Asp Leu Phe Val Ile Arg Asn Ala
 130 135 140
 Gly Asn Ile Ile Pro Pro Tyr Gly Ala Ala Asn Gly Gly Glu Gly Ala
 145 150 155 160
 Ala Met Glu Tyr Ala Leu Val Ala Leu Glu Ile Asn Gln Ile Ile Val
 165 170 175
 Cys Gly His Ser His Cys Gly Ala Met Lys Gly Leu Leu Lys Leu Asn
 180 185 190
 Ser Leu Gln Glu Lys Leu Pro Leu Val Tyr Asp Trp Leu Lys His Thr
 195 200 205
 Glu Ala Thr Arg Arg Leu Val Leu Asp Asn Tyr Ser His Leu Glu Gly
 210 215 220
 Glu Asp Leu Ile Glu Val Ala Val Ala Glu Asn Ile Leu Thr Gln Leu
 225 230 235 240
 Lys Asn Leu Gln Thr Tyr Pro Ala Ile His Ser Arg Leu His Arg Gly
 245 250 255
 Asp Leu Ser Leu His Gly Trp Ile Tyr Arg Ile Glu Glu Gly Glu Val
 260 265 270
 Leu Ala Tyr Asp Gly Val Leu His Asp Phe Val Ala Pro Gln Ser Arg
 275 280 285
 Ile Asn Ala Leu Glu Pro Glu Asp Glu Tyr Ala Pro His Pro Asn Ser
 290 295 300
 Pro Leu Ile Ser Tyr Asp Ala Phe Lys Val Pro Gly Lys Glu Arg Pro
 305 310 315 320
 Gly Arg Glu Lys Ala Thr Glu Ser Pro Ala Pro Gln Leu Ser Pro Leu
 325 330 335
 Pro Gly Phe Gly His Leu Pro Arg Glu Gln Ala Glu Arg Ile Tyr Arg
 340 345 350
 Gly Ser Arg Lys Leu Ala Ala Ala Leu Glu His His His His His His
 355 360 365

<210> SEQ ID NO 10
 <211> LENGTH: 1107
 <212> TYPE: DNA
 <213> ORGANISM: Synechocystis PCC6803
 <220> FEATURE:
 <221> NAME/KEY: gene
 <222> LOCATION: (1)..(1107)
 <223> OTHER INFORMATION: carbonic anhydrase (periplasmic expression)

<400> SEQUENCE: 10

atgaacaata acgatctctt tcaggcatca cgtcggcggtt ttctggcaca actcggcggc	60
ttaaccgtcg ccgggatgct ggggccgtca ttgttaacgc cgcgacgtgc gactgcggcg	120
caagcggcca tgggagccga agtttcattg atatccaga caaattgccc cgctgtccta	180
gacccccgc cgcaagtctt caatgataca atggaaggct ggaattccat caacggcgat	240
cgtatgcaaa gactcatoga gggactacaa aaatttcgag aaggttatct ctcttccac	300
cgtgacctct ttgagcaact ttctcaggc caacatcccc gcattctctt catctgttgt	360
tccgattccc ggggtgaccc caatttaatc acccaatcgg aagtggcgga cctgtttgtt	420

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attcgcaacg ctggcaatat tatteccccc tatggagcag ccaacgggtg ggaaggggca 480
gccatggaat atgccctagt ggcgctggaa attaatcaga tcategtctg tggccattcc 540
cactgcgag ccatgaaagg tctgctcaaa ctcaactctc tccaggaaaa acttcctctg 600
gtgtacgatt ggctcaaaca tacggaagcc acccgccgtc tagttctaga caattacagc 660
catctggaag gggaagatgt gattgaagtt gctgtggcag aaaatattct caccctaactc 720
aaaaacctcc agacctatcc cgccatccat tcccggttac atcggggaga cctttccctc 780
cacggctgga tttatcgcat tgaagagggt gaagtactgg cctacgacgg tgtactccat 840
gattttgtcg ccccccaaag tcgcatcaat gccctggagc cggaggatga gtacgtcccc 900
catcccaact caccctgat ttctacgat gcgtttaagg ttcccgga ggaacgtcct 960
ggctgtgaga aagcaacaga atccccagct ccccaactgt ctctttacc tggttttggc 1020
catttgccca gggaacaggc ggagcgcat tatcgaggct cccgtaagct tgcggccgca 1080
ctcgagcacc accaccacca cactga 1107

```

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<210> SEQ ID NO 11
<211> LENGTH: 231
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(231)
<223> OTHER INFORMATION: carbonic anhydrase (cytoplasmic expression)

```

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<400> SEQUENCE: 11

```

```

Met Lys Glu Ile Ile Asp Gly Phe Leu Lys Phe Gln Arg Glu Ala Phe
  1             5             10            15
Pro Lys Arg Glu Ala Leu Phe Lys Gln Leu Ala Thr Gln Gln Ser Pro
  20            25            30
Arg Thr Leu Phe Ile Ser Cys Ser Asp Ser Arg Leu Val Pro Glu Leu
  35            40            45
Val Thr Gln Arg Glu Pro Gly Asp Leu Phe Val Ile Arg Asn Ala Gly
  50            55            60
Asn Ile Val Pro Ser Tyr Gly Pro Glu Pro Gly Gly Val Ser Ala Ser
  65            70            75            80
Val Glu Tyr Ala Val Ala Ala Leu Arg Val Ser Asp Ile Val Ile Cys
  85            90            95
Gly His Ser Asn Cys Gly Ala Met Thr Ala Ile Ala Ser Cys Gln Cys
 100           105           110
Met Asp His Met Pro Ala Val Ser His Trp Leu Arg Tyr Ala Asp Ser
 115           120           125
Ala Arg Val Val Asn Glu Ala Arg Pro His Ser Asp Leu Pro Ser Lys
 130           135           140
Ala Ala Ala Met Val Arg Glu Asn Val Ile Ala Gln Leu Ala Asn Leu
 145           150           155           160
Gln Thr His Pro Ser Val Arg Leu Ala Leu Glu Glu Gly Gly Ser Leu
 165           170           175
His Gly Trp Val Tyr Asp Ile Glu Ser Gly Ser Ile Ala Ala Phe Asp
 180           185           190
Gly Ala Thr Arg Gln Phe Val Pro Leu Ala Ala Asn Pro Arg Val Cys
 195           200           205
Ala Ile Arg Leu Arg Gln Pro Thr Ala Ala Lys Leu Ala Ala Ala Leu
 210           215           220

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Glu His His His His His His
225 230

<210> SEQ ID NO 12
<211> LENGTH: 696
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli
<220> FEATURE:
<221> NAME/KEY: gene
<222> LOCATION: (1)..(696)
<223> OTHER INFORMATION: carbonic anhydrase (cytoplasmic expression)

<400> SEQUENCE: 12

```
atgaaagaga ttattgatgg attccttaaa ttccagcgcg aggcatttcc gaagcgggaa    60
gccttggtta aacagctggc gacacagcaa agcccgcgca cactttttat ctctgctccc    120
gacagccgctc tggtcctga gctggtgacg caacgtgagc ctggcgatct gttcgttatt    180
cgcaacgcggg gcaatatcgt cccttcctac gggccggaac cgggtggcgt ttctgcttcg    240
gtggagtatg ccgtcgctgc gcttcgggta tctgacattg tgatttgtgg tcattccaac    300
tgtggcgcgca tgaccgccat tgccagctgt cagtgcattg accatatgcc tgccgtctcc    360
cactggctgc gttatgccga ttcagccgcg gtcgttaatg aggcgcgccc gcattccgat    420
ttaccgtcaa aagctgcggc gatggtacgt gaaaacgtca ttgctcagtt ggctaatttg    480
caaaactcacc catcggtgcg cctggcgctc gaagagggcg gatcgctgca cggctgggtc    540
tacgacattg aaagcggcag catcgagctc ttgacggcg caaccgcga gtttgtgcca    600
ctggccgcta atcctcgctt ttgtgccata cgcctacgcc aaccgaccgc agcgaagctt    660
gcggccgcac tcgagcacca ccaccaccac cactga                                696
```

<210> SEQ ID NO 13
<211> LENGTH: 275
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(275)
<223> OTHER INFORMATION: carbonic anhydrase (periplasmic expression)

<400> SEQUENCE: 13

```
Met Asn Asn Asn Asp Leu Phe Gln Ala Ser Arg Arg Arg Phe Leu Ala
 1           5           10          15

Gln Leu Gly Gly Leu Thr Val Ala Gly Met Leu Gly Pro Ser Leu Leu
 20          25          30

Thr Pro Arg Arg Ala Thr Ala Ala Gln Ala Ala Met Gly Lys Glu Ile
 35          40          45

Ile Asp Gly Phe Leu Lys Phe Gln Arg Glu Ala Phe Pro Lys Arg Glu
 50          55          60

Ala Leu Phe Lys Gln Leu Ala Thr Gln Gln Ser Pro Arg Thr Leu Phe
 65          70          75          80

Ile Ser Cys Ser Asp Ser Arg Leu Val Pro Glu Leu Val Thr Gln Arg
 85          90          95

Glu Pro Gly Asp Leu Phe Val Ile Arg Asn Ala Gly Asn Ile Val Pro
100          105          110

Ser Tyr Gly Pro Glu Pro Gly Gly Val Ser Ala Ser Val Glu Tyr Ala
115          120          125

Val Ala Ala Leu Arg Val Ser Asp Ile Val Ile Cys Gly His Ser Asn
130          135          140
```


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Cys Gly Ala Met Thr Ala Ile Ala Ser Cys Gln Cys Met Asp His Met
 145 150 155 160
 Pro Ala Val Ser His Trp Leu Arg Tyr Ala Asp Ser Ala Arg Val Val
 165 170 175
 Asn Glu Ala Arg Pro His Ser Asp Leu Pro Ser Lys Ala Ala Ala Met
 180 185 190
 Val Arg Glu Asn Val Ile Ala Gln Leu Ala Asn Leu Gln Thr His Pro
 195 200 205
 Ser Val Arg Leu Ala Leu Glu Glu Gly Gly Ser Leu His Gly Trp Val
 210 215 220
 Tyr Asp Ile Glu Ser Gly Ser Ile Ala Ala Phe Asp Gly Ala Thr Arg
 225 230 235 240
 Gln Phe Val Pro Leu Ala Ala Asn Pro Arg Val Cys Ala Ile Arg Leu
 245 250 255
 Arg Gln Pro Thr Ala Ala Lys Leu Ala Ala Ala Leu Glu His His His
 260 265 270
 His His His
 275

<210> SEQ ID NO 14
 <211> LENGTH: 828
 <212> TYPE: DNA
 <213> ORGANISM: Escherichia coli
 <220> FEATURE:
 <221> NAME/KEY: gene
 <222> LOCATION: (1)..(828)
 <223> OTHER INFORMATION: carbonic anhydrase (periplasmic expression)

<400> SEQUENCE: 14

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caagcggcca tgggaaaaga gattattgat ggattcctta aattccagcg cgaggcattt	180
ccgaagcggg aagccttggt taaacagctg gcgacacagc aaagcccgcg cacacttttt	240
atctcctgct ccgacagccg tctgggtcct gagctgggtga cgcaacgtga gcctggcgat	300
ctgttcgtta ttgcgaacgc gggcaatc gtccttcct acggggcgga acccggtggc	360
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ggtcattcca actgtggcgc gatgaccgcc attgccagct gtcagtgcac ggaccatatg	480
cctgccgtct cccactggct gcgttatgcc gattcagccc gcgtcgtaa tgaggcgcgc	540
ccgcattccg atttaccgtc aaaagctgcg gcgatggtag gtgaaaacgt cattgctcag	600
ttggctaatt tgcaaaccca tccatcggtg cgcctggcgc tcgaagaggg cggtatcgctg	660
cacggctggg tctacgacat tgaaagcggc agcatcgag cttttgacgg cgcaaccgcg	720
cagtttgtgc cactggcgcg taatcctcgc gtttgtgcca tacgcctacg ccaaccgacc	780
gcagcgaagc ttgcggcgcg actcagagcac caccaccacc accactga	828

<210> SEQ ID NO 15
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Forward (No signal) : NCA-C-FP

<400> SEQUENCE: 15

catatgcacg gcaatcacac c	21
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<210> SEQ ID NO 16
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Common reverse : NCA-BP

<400> SEQUENCE: 16
aagcttttca ataactacac gtgcatt 27

<210> SEQ ID NO 17
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward (No signal) : SCA-C-FP

<400> SEQUENCE: 17
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<210> SEQ ID NO 18
<211> LENGTH: 29
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Common reverse : SCA-BP

<400> SEQUENCE: 18
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<210> SEQ ID NO 19
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward (No signal) : ECA-C-FP

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<210> SEQ ID NO 20
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<212> TYPE: DNA
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<400> SEQUENCE: 21
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<210> SEQ ID NO 22
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<210> SEQ ID NO 23

<211> LENGTH: 27

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<220> FEATURE:

<223> OTHER INFORMATION: Forward (Signal) : ECA-T-FP

<400> SEQUENCE: 23

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<210> SEQ ID NO 24

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Forward (signal) : NCA-INPNC-FP

<400> SEQUENCE: 24

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27

<210> SEQ ID NO 25

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Reverse : NCA-INPNC-BP

<400> SEQUENCE: 25

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27

The invention claimed is:

1. A composition for capturing carbon dioxide, the composition comprising at least one selected from the group consisting of:

an isolated host cell transformed with a vector comprising a heterologous nucleic acid encoding a recombinant carbonic anhydrase;

a cell lysate of the isolated host cell, or a fraction thereof; and

a carbonic anhydrase isolated from the isolated host cell, wherein the carbonic anhydrase has the amino acid sequence of SEQ ID NO: 3.

2. The composition as claimed in claim 1, wherein the isolated host cell has an expression of carbonic anhydrase in periplasmic space.

3. The composition as claimed in claim 1, wherein the fraction of the cell lysate includes an isolated host cell fraction, a soluble fraction, an insoluble fraction, or a periplasmic fraction of the cell lysate.

4. The composition as claimed in claim 1, wherein the carbonic anhydrase is obtained from *Neisseria gonorrhoea*.

5. The composition as claimed in claim 1, wherein the nucleic acid encoding the carbonic anhydrase has the nucleic acid sequence of SEQ ID NO: 4.

6. The composition as claimed in claim 1, wherein the isolated host cell is *Escherichia coli*.

7. A composition for converting carbon dioxide to a carbonate or a bicarbonate, the composition comprising:

the composition as claimed in claim 1; and

a metal cation.

8. The composition as claimed in claim 7, wherein the metal cation is Na⁺, Ca²⁺, Fe²⁺, Mn²⁺, Sr²⁺, Ca²⁺, Ba²⁺, Zn²⁺, or Pb²⁺; or a nitrate, hydrochloride, hydrate or solution thereof.

9. The composition as claimed in claim 7, wherein the carbonate is sodium carbonate, calcium carbonate, iron carbonate, manganese carbonate, strontium carbonate, barium carbonate, zinc carbonate, or lead carbonate.

10. A method for capturing carbon dioxide comprising: preparing the carbon dioxide capturing composition as claimed in claim 1; and feeding carbon dioxide into the carbon dioxide capturing composition.

11. A method for converting carbon dioxide to a carbonate or a bicarbonate comprising:

(a) preparing the carbon dioxide capturing composition as claimed in claim 1; and

(b) feeding a metal cation and carbon dioxide into the carbon dioxide capturing composition.

12. The method as claimed in claim 11, wherein the step (b) of feeding a metal cation and carbon dioxide into the carbon dioxide capturing composition comprises:

feeding a metal cation and carbon dioxide in sequence, feeding carbon dioxide and a metal cation in sequence, or feeding a metal cation and carbon dioxide at once.

13. The method as claimed in claim 11, wherein the metal cation is Na⁺, Ca²⁺, Fe²⁺, Mn²⁺, Sr²⁺, Ca²⁺, Ba²⁺, Zn²⁺, or Pb²⁺; or a nitrate, hydrochloride, hydrate or solution thereof.

14. The method as claimed in claim 11, wherein the carbonate is sodium carbonate, calcium carbonate, iron carbonate, manganese carbonate, strontium carbonate, barium carbonate, zinc carbonate, or lead carbonate.

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